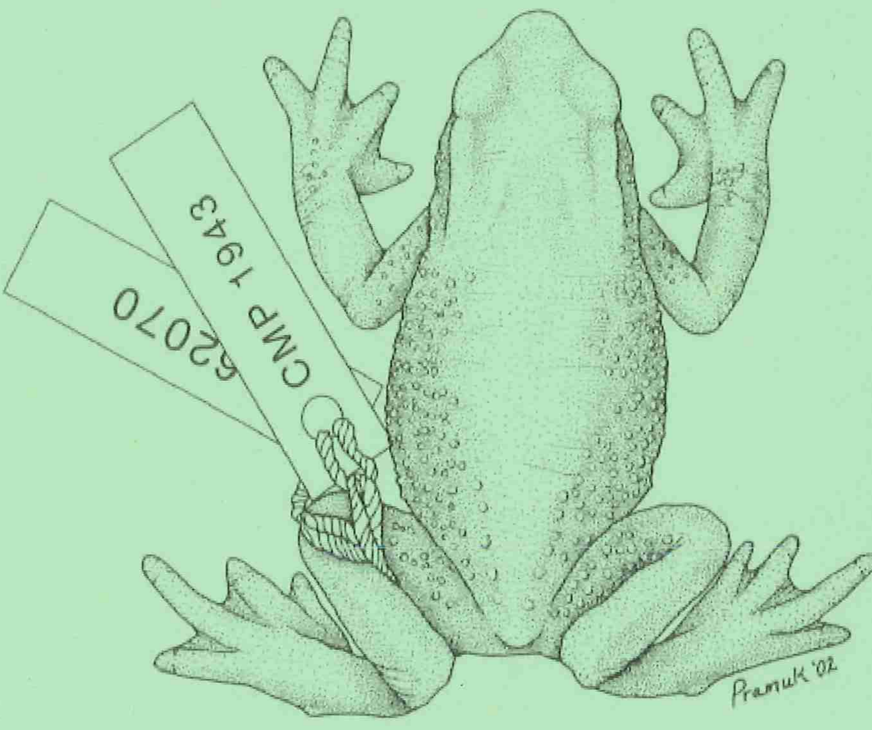
Herpetological Collecting and Collections Management

Revised Edition



John E. Simmons

Society for the Study of Amphibians and Reptiles

SOCIETY FOR THE STUDY OF AMPHIBIANS AND REPTILES

HERPETOLOGICAL CIRCULAR NO. 31

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Cover : The cover drawing is an *Atelopus ignescens*, or jambato, from east of San Miguel de Salcedo in Napo Province, Ecuador (3660 m). The specimen was collected by John Simmons and David Hillis on 25 March 1984. Once jambatos were one of the most common anurans in the Northern Andes. The species is now extinct, and can only be studied by using preserved and documented specimens in museum collections.

The artist is Jennifer B. Pramuk. She is a PhD student at KU, writing a dissertation on the Evolution of New World *Bufo*. She can be reached at Natural History Museum, University of Kansas, 1345 Jayhawk Boulevard, Lawrence, KS 66045-7561 USA

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INTRODUCTION

This book is for anyone who needs to collect and preserve amphibians and reptiles, or who works with preserved specimens. If field workers understand how specimens are processed and used in museums, they will prepare better specimens. If collection users understand how animals are collected and preserved in the field, they will make better use of the specimens. If all of us understand how collections are managed, specimens will be better utilized and preserved for the future.

Many changes in fieldwork practices and in collection management have taken place since the publication of the first edition of this book in 1987. Two other publications have addressed herpetological collecting and collections (Casas-Andreu et al. 1991, Scrocchi and Kretzschmar 1996). Collecting has become much more complex worldwide—fewer natural areas are left where amphibians and reptiles may be collected, and it is more difficult and time consuming to obtain permits to collect. Many amphibian populations have declined drastically in numbers or have gone extinct. At the same time, properly collected, preserved, and managed collections have increased in research value. The variety of research using preserved collections has increased, as has the number of collection users. The data associated with collections are in high demand for bioinformatic and conservation research (e.g., McCarthy 1998).

Since the first edition of this book appeared, standards for specimen documentation (Garrett 1989) and collection care (Society for the Preservation of Natural History Collections 1994) have been established. Because every herpetological collection has its own peculiarities, strengths, and weaknesses, the intent of the first edition was to provide "a framework for standardizing curatorial practices among diverse collections..." (Simmons 1987). I hope that this revised edition will provide an even better, but equally flexible framework for collection care in line with recognized professional standards and practices.

This book is intended only as an introduction to collecting and collection management. The development of techniques as diverse as molecular systematics (Hillis et al. 1996), collection risk assessment (Waller 1995), preservation and management assessments (McGinley 1993, Williams et al. 1996), and the application of the concept of preventive conservation to natural history collections (Rose and Hawks 1995, Williams 1999) have made the care and management of collections far more sophisticated than can be covered in just one volume.

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I thank William E. Duellman and Linda Trueb for the opportunity and encouragement to develop my ideas about how specimens should be collected and how collections should be managed. I am also indebted to the numerous colleagues who have taken the time to tell me about their special techniques and procedures for collecting and preserving amphibians and reptiles.

My ideas about fluid preservation, collection care, and collection management have evolved enormously since 1987. While it is impossible to mention everyone who has helped me to shape and refine my ideas, I would particularly like to acknowledge Paisley Cato, Andries van Dam, Catharine Hawks, Elizabeth Merritt, Carolyn Rose, Sally Shelton, Robert Waller, and Stephen Williams. Special thanks go to the students in my *Introduction to Museum Collection Management* classes in the Museum Studies Program at the University of Kansas, and to Yaneth Muñoz-Saba and the participants in the *Cuidado y Manejo de Colecciones de Historia Natural* workshops in Colombia, El Savador, Ecuador and Mexico both for their inspiration and for persisting in asking difficult questions about collection care and management.

Lastly, I thank my wife, Ligia Galarza Simmons, who has cheerfully tolerated long absences while I was out of the country, patiently critiqued my ideas, and stood by me throughout my professional career.

PART I. A BRIEF HISTORY OF SYSTEMATIC COLLECTIONS AND SPECIMEN PREPARATION

> The failure to apply scientific methodology to preservation practices is particularly intriguing in a setting where scientific methodology prevails...very few studies in natural history have applied the scientific method to evaluate preservation treatments.
> —S.L. Williams (1999)

Natural history collections have played an important, though often overlooked role in the history of science. The available preservation technology has determined what specimens could be kept in collections, and those collections in turn have driven the development of our scientific knowledge of the world (Asma 2001, Whitehead 1970, 1971). An understanding of how collections have evolved is fundamental to knowing how to manage them properly.

Collecting things is a universal human trait. Natural history specimens were some of the first objects to be collected in museums (de Borhegyi and Hanson 1982, Pearce 1992, Asma 2001). As the art of magic grew into the practice of science in the western world, odd collections of miscellaneous objects gradually became the ordered systematic resources that now play an integral role in our interpretation of natural phenomenon. Today there are 2.5 to 3 billion natural history specimens preserved worldwide in 6,500 museums (Duckworth et al. 1993, Mares 1993). This is far more elements than in collections of art or history museums. Unlike other kinds of museum collections, most natural history specimens are collected to be used for research—research affects both collection growth and how collections are managed and cared for. Worldwide, the ratio of natural history collection care workers to specimens is about 1:200,000, which means that these resources must be carefully managed at the collection level.

The history of preservation predates the history of museums. Dehydration, in the form of mummification, is the oldest method of intentional preservation. Mummies were made at least 7,800 years ago in Peru and at least 5,000 years ago in Egypt (Brier 1998). These examples are the earliest known attempts to keep animal and plant specimens permanently. Collections of "curious objects" were assembled by such august figures as Solomon of Jerusalem and Augustus of Rome. Tuthmosis III (1504-1450 BC) of Egypt had an extensive collection of flora and fauna from Asia. Nebuchadrezzar (605-562 BC) kept large collections, including natural history specimens, in Babylon (Rigby and Rigby 1944).

The development of natural history museums can be divided into six periods (after Whitehead 1970):

I. Greco-Roman	to 400 CE
II. Pre-Renaissance	400 - 1400
III. Renaissance	1400 - 1600
IV. Pre-Linnean	1600 - 1750
V. Linnean	1750 - 1850
VI. Post-Linnean	1850 - present

Greco-Roman, to 400 CE

Aristotle (384-322 BCE) developed a system of classification of organisms that endured for a very long time. A good part of Aristotle's knowledge of animals came from his own dissections and observations of specimens (French 1994). He recognized approximately 540 species of animals, arranged in a single, graded progression or ***scala natura***.

Aristotle started the long tradition of sending one's students into the field to collect specimens from obscure localities (Flower 1898), a custom that continues today. His student, Alexander the Great, collected interesting specimens he found while conquering the world. For example, Aristotle's description of the elephant in *Historia animalium* reveals that he had direct knowledge of Indian elephants from information obtained by Alexander (French 1994).

If we define a **museum** as a place where *learning* and *objects* are combined, then the first museum was the Temple of the Muses founded in the 3rd century BCE in Alexandria (Egypt) by Ptolemy Sotor (305-283 BCE) (Alexander 1979, French 1994, Simpson 1998, Singer 1959). A former student of Aristotle, Demetrius, served as an advisor to Ptolemy, and helped create the museum. It continued to flourish under Ptolemy Philadelphius (285-246 BCE) with a library of 500,000 books as well as collections of art and natural history. Natural history collecting was widespread by this time—for example, an exotic sea shell collection was found in the ruins of Pompeii (French 1994). During this period, preservation technology was limited to dehydration (Singer 1959, Whitehead 1970).

Pre-Renaissance, 400 - 1400

The history of modern science began in China and Arabia with the development of alchemy, which reached Europe via Alexandria and other centers of learning in Hellenistic Egypt (Holmyard 1990). Alchemy had both a practical and an esoteric side. It was, in essence, the search for a substance called the *philosopher's stone* that could be used to turn base metals into precious metals. The work of the early Islamic scholars reached Europe in the 12th and 13th centuries when the Arabic texts were translated into Latin (Holmyard 1990). Between 1200 and 1225, Aristotle's works were also recovered and translated into Latin. These translations initiated the revival of learning in Europe, which resulted in the appearance of the first cabinets of curiosity.

Cabinets of curiosity began as cabinets, privately owned by rich collectors, filled with treasures of nature and other objects. The cabinets grew to occupy whole rooms and eventually many became museums. Henry, the Duke of Saxony (1129-1195), had a griffin's claw from Palestine in his collection. Lorenzo the Magnificent (1449-1492) had a unicorn horn in his (Hooper-Greenhill 1992). The first recorded use of the word *museum* was in reference to Lorenzo's collection (Impey and MacGregor 1985, Murray 2000).

No records exist of attempts to develop preservation technologies to keep specimens in these early collections other than by dehydration, and there was no systematic organization of the collections. It was from the spirit of "the veneration of the rare, the unusual, the wonderful and the miraculous" that modern museums arose (Whitehead 1970). Goode (1889) suggested that apothecaries were the antecedents of the modern curator, referring to the description of the apothecary shop found in Shakespeare:

> "...Meager were his looks,
> Sharp misery had worn him to the bones;
> And in his needy shop a tortoise hung,
> An alligator stuffed, and other skins
> Of ill-shaped fishes..."
> (*Romeo and Juliet*, Act V, Scene I)

Even then adequate funding to maintain the facility ("needy shop") was a problem.

Renaissance, 1400 - 1600

During the Renaissance, the invention of printing played an important role in the advancement of scientific knowledge. Between 1469 and 1499, at least 39 editions of Pliny's *Natural History* and 11 editions of Aristotle's works were published. Cabinets of curiosity blossomed across Europe, as observation and experimentation became established methods of scientific study (Singer 1997). Collectors continued to arrange their specimens based on Aristotle's *scala natura*.

In this era the first great collectors appeared. Konrad Gesner (1516-1565) and Ulyssis Aldrovandi (1522-1605) were both important natural history writers who had private museums (Murray 2000, Nordenskiöld 1949). Aldrovandi was a professor of medicine in Padua and Rome who spent his fortune collecting animals and hiring artists to draw them. It took him fifty years to get the first volume of his masterwork ready for publication, but it stood as the most comprehensive zoology text until the 18th century. Gesner was born in Zurich. His *Historia Animalium* (a work of 3,500 pages in four immense folio volumes) arranged all known animal species using the principles of Aristotle, with an illustration of each. Gesner included many previously unknown animals such as the opossum, bird of paradise, sloth, guinea pig, and armadillo (Ashworth 1991, Impey and MacGregor 1985).

During the Renaissance the first serious attempts at developing preservation technology began, and collections were described and cataloged. Specimens from several of these Renaissance era cabinets of curiosity have survived (e.g., some of Gesner's specimens are in a museum in Basel). Natural history collecting was now done with a scientific purpose. In 1565, a Flemish collector wrote that ideally, collections should represent a systematic classification of all materials in the universe.

Pre-Linnean, 1600 - 1750

During this period, natural history museums proliferated and expanded. Cabinets of curiosity became indispensable for preserving the artifacts of history and the taxonomy of the natural world (Orosz 1990). Collections changed from random catalogs of nature to collections made for the purpose of understanding and classifying nature. The mid-17th century saw the rise of taxidermy and a number of other advances in preservation technology that aided the development of museums. These included arsenic and mercuric chloride as pesticides, colored wax and mercury anatomical injections, and the discovery that specimens could be preserved in alcohol (Whitehead 1971). In 1727, Casper F. Neickel published *Museographia*, which addressed problems of classification, collection care, and sources of specimens (Alexander 1979).

The knowledge of how to produce alcohol by fermentation is very old (Forbes 1948)—there is a 6,000 year old recipe for beer from Iraq (Michel et al. 1992). But fermentation can only produce alcohol of 12-15%, because the alcohol kills the yeast. Distillation is required to make stronger alcohol. Distillation was developed in the Middle East or in Africa (Holmyard 1990). Double-rimmed extraction pots which could have distilled alcohol were being used as early as 3000 BCE in Mesopotamia (Brock 1993).

Distillation was introduced to the West by the writings of the alchemist Zosimus, in the 3rd century CE (Holmyard 1990). Distillation is based on evaporation, and was used by alchemists to try and refine the essence, or the spirit, of a substance, which is why alcohol is also called *spirit of wine* or just *spirits* (Forbes 1948). The word alcohol comes from the Arabic *al kohl*, which was a fine, black powder used as eye shadow. The fine powder was considered to be an *essence*, or spirit, of an element (Holmyard 1990).

Another advance in preservation technology came about in 1615 when the English Crown issued a decree that gave the Royal Navy priority use of wood for shipbuilding, forcing glass furnaces to switch to coal. Coal made hotter fires, so lead oxide could be added to the glass to make an inexpensive, clear "flint glass" with fewer bubbles (Frank 1982, Singer 1959). Things placed inside these vessels could be seen without having to break the seal and open the container.

Preservation of natural history specimens in alcohol can be traced with accuracy to 1662, the year that William Croone showed the Royal Society of London two puppies preserved in "spirit of wine" in a hermetically sealed glass vessel (Birch 1968, Cole 1944). The preservative was about 67% ethyl alcohol, distilled from fermented barley mash.

Collection care at this time was still not terribly good. According to Barber (1980), "...William Swainson compared the storerooms [of the British Museum] with the catacombs at Palermo, each of which was apparently opened once a year to determine how much decay had occurred and to deposit fresh material."

As preservation technology improved, and specimens poured in to Europe from all over the world, collections became scientifically more useful, but were still largely organized according to the principles of Aristotle.

Linnean, 1750 - 1850

During this period modern systematic museums appeared in Europe and the Americas, corresponding to the increase in scientific exploration and collecting worldwide (Singer 1997).

Modern zoological nomenclature dates from the publication of the 10th edition of *Systema Naturae* in Sweden by Carl von Linné (1707-1778), in 1758. Linné subsequently Latinized his name to Linnaeus. As a student, he made extensive plant and anthropology collections in Sweden and Lapland. As a professor of botany at Upsala University, he sent his students on frequent collecting expeditions. It wasn't easy to be a student of Linnaeus—some accounts say that as many as a third of his students died on these expeditions (Nordenskiöld 1949).

The Linnean system of nomenclature quickly became the organizing principle for natural history collections. Because Linnaeus thought that all species had been created at one time, collections were *typological* (with one or two specimens to represent each species), based on the *scala natura* concept of Aristotle. Rather than continuing to collect things such as "...the Frightful large bat" or "Mermaid's hand," or "the skeleton and stuffed skin of a woman who had eighteen husbands...," all of which are listed in the catalog of the Oxford Museum in England (Whitehead 1971), the Linnean classification system brought order to museum collections. The ability to *arrange collections in order* stimulated the search for more species. Greater emphasis was placed on collecting and preserving specimens, and collections were cataloged, systematically arranged, and reported in the scientific literature.

James Petiver (1658-1718) of Aldersgate in London printed up a sheet of instructions for preserving animals in "Rack, Rum or Brandy" and urged travelers to collect for him (Stearns 1953). Several improvements in methods of preservation were now in use. A paper published in 1748 listed the four principal techniques for specimen preservation. These were (1) stuffing skins and allowing them to dry (no preservatives); (2) putting specimens in spirit of wine; (3) embalming specimens with spices, salt, alum, or lime; and (4) oven drying specimens (Reamur 1748). All four of these methods are still in use today.

Fluid preservation was gaining in popularity, although it was still too expensive for widespread use. Thomas Pole wrote that "Preparations of almost every part are occasionally kept in spirits…as by this mode they undergo less change of appearance than by any other method of preservation, and consequently give the best idea of the natural or diseased appearance; but the expensiveness of the glass and spirits is a great inducement to the making of so many dry preparations" (Pole 1790). Although few dry specimens from this era remain in collections, some fluid preserved specimens do. For example, two birds preserved in alcohol in 1779 by a naturalist on Cook's voyage to Hawaii are still usable specimens (Burton 1969).

The standards for fluid preservation varied widely. In 1840, Spencer Fullerton Baird, then just 15 years old, wrote to the famous John James Audubon to describe a bird he had shot but was unable to identify. Audubon wrote back with a request for specimens, instructing young Baird, "Please to collect all the Shrews, Mice (field or wood), rats, bats, Squirrels, etc., and put them in a jar with common Rum, not whiskey, brandy, or alcohol. All of the latter spirits are sure to injure the subjects..." (Herrick 1917).

Post-Linnean, 1850 - present

In 1859, the publication of *On the Origin of Species* by Charles Darwin started a fundamental revolution in biology that had a tremendous effect on how specimens were collected, preserved, stored, and exhibited (Conn 1998, Hooper-Greenhill 1992, Whitehead 1971). In the post-Linnean period museums stopped displaying all of their collections to the public, as synoptic displays gave way to naturalistic displays. These new exhibits showed specimens in an environmental context. Collecting became systematic and comprehensive as the need to include the variation within species in collections was recognized; collections became archives of the natural world.

The Future of Systematic Collections

As the concept of what a museum is has evolved, so has the concept of what constitutes a collection (Pyenson and Sheets-Pyenson 1999). The concept of collection care and curation has undergone a major revolution during the last 150 years (Ford and Simmons 1997). The nature of collections is undergoing a revolution that places increasing responsibility on professional collection management (Simmons 1991, 1993; Williams 1999). Once, the specimen itself and a general geographic provenance were considered sufficient for the accession of material. As our abilities to analyze and comprehend the natural world have evolved, our requirements for specimens and data have become more exacting. The importance of the individual specimen as a voucher is one fairly recent development (Lee et al. 1982). The minimum requirements for locality data are far more specific that they once were. Data regarding the relationship of the individual specimen and its habitat are becoming as valuable as the specimen itself. A modern systematic herpetology collection contains not just preserved specimens, but histological preparations, tissue samples, field notes, tape recordings of vocalizations, images of specimens in life, images of habitat, maps, and bibliographic references. All these various components of the systematic collection must be linked together and carefully managed to advance scientific research.

If the history of natural history collections tells us anything, it is that the future will bring new and creative uses for collections. As recently as 20 years ago, it was thought that the DNA contained in museum specimens was nearly useless. It is now routinely extracted for research. Similar sorts of biochemical and other research uses of specimens will be developed in the future. For many years the primary function of systematic collections was amassing large numbers of specimens for the purpose of naming large numbers of species. However, the research based on collections has expanded considerably in breadth. With proper documentation, preserved collections provide baseline data for ecological studies. Field notes usually contain a wealth of information on the conditions under which specimens were obtained. Parameters related to the ecology of a species that are difficult or impossible to measure in the field (e.g., food selection and prey size, reproductive condition) are studied more easily and accurately with preserved material. New ways of using data associated with specimens will be discovered. The field of biodiversity informatics is one example—using the specimen records from multiple natural history collections, satellite images, landform and climate data, sophisticated predictive computer models are being made (Krishtalka and Humphrey 2000).

The value of systematic collections as scientific resources will continue to increase. Collection growth will slow owing to worldwide environmental changes and as increasing human populations expand into previously undeveloped areas. Areas where specimens may be collected will become fewer and fewer as natural areas of the earth are degraded by the activities of humans. Fieldwork will continue to become more complicated and more costly as travel expenses rise and documentation and permit requirements increase.

The role of well-managed systematic collections in better understanding the natural world will certainly continue to expand.

PART II. FIELD COLLECTING

> Lucky are those who travel without instruments that break, without dried plants that get wet, without animal collections that rot; lucky are those who travel the world to see it with their own eyes, trying to understand it, and recollecting the sweet emotions that nature inspires.
> —Alexander von Humboldt, 1889

> The lizard can be caught with the hand, yet it is found in king's palaces.
> —Proverbs 20:28

Standards for field collecting have become more sophisticated to meet the demands of the evolving fields of systematics and ecology. As a result, more is expected of a field collector than ever before, yet opportunities to learn good field methods are becoming scarce as the number of good field collectors decreases. This section focuses on techniques and general recommendations for collecting, preserving, and documenting reptiles and amphibians in the field.

FIELD TRIP PLANNING

Proper planning is critical to the success of fieldwork. Proper planning will insure that you are not caught unprepared by lack of equipment, climate, or geographic surprises. Planning begins with allowing plenty of time to obtain the necessary permits to do the work. The process of applying for and receiving permits may take months to a year or more. Requirements vary greatly from place to place, and usually call for a description of the proposed project or a formal research proposal (in the appropriate language if in a foreign country). Supplies and equipment must be carefully evaluated, and orders placed well in advance of the planned collecting trip.

Permits

Almost every place on the planet where amphibians and reptiles can be caught now requires the collector to obtain legal permission before collecting. Within the United States, federal, state, or local authorities may require permits. To import specimens that are collected outside the United States, the collector must be able to show legal permission to possess them and to export them from their country of origin. Refer to *Appendix I* for information on obtaining permits. Table 1 lists the main types of permissions, permits, and declarations necessary to obtain specimens and import them into the United States.

In general, permission must be obtained to collect specimens in the wild, to remove them from a foreign country, and to import them in to the United States (Malaro 1998, Tompkins 1998). Some species are protected by special legislation, such as CITES (Convention on International Trade in Endangered Species) or the ESA (Endangered Species Act). These protected species may only be imported into the United States through a US Fish and Wildlife Designated Port (see *Appendix I*), unless a Designated Port Exemption Permit is obtained. The regulations governing collecting, export, import, and possession of amphibians and reptiles are complex (Duellman 1999, Tompkins 1998). In particular, the Lacey Act (Malaro 1998, Tompkins 1998) requires compliance with all wildlife laws (both in the United States and in other countries) by requiring that any specimen imported into the United States has been legally obtained in its country of origin.

Table 1. Types of permissions, permits and declarations for collecting.

Type of permission, permit, or declaration	Purpose
Collecting permit	Legal permission for a collector to possess specimens taken from the wild. Generally, collecting permits specify the number of specimens of each species one is allowed to collect, and how the collection will be divided. Collecting permits may be issued at the local, state, regional or federal level depending on the country.
Landowner's permission to collect	An oral or written agreement with a private landowner or government to collect specimens from a particular place.
Export permit	Permission to remove legally obtained specimens from their country of origin.
CITES permit	Required to export or import CITES listed species, or move them from one institution to another once they are accessioned (see Appendix I).
3-177 declaration	Declaration form that must be filed with the US Fish and Wildlife service to notify them that specimens were imported to or exported from the United States. This form must be filed whenever animals or animal parts (e.g., tissue samples, skins, or teeth) are transported in or out of the United States.
Designated Port Exemption Permit	Required to bring controlled wildlife through a non-designated port of entry into the United States (see Appendix I).

Steps in Planning Field Work

1. Determine where you want to collect, what you want to collect, and how many individuals of each species are needed.
2. Make arrangements to deposit the specimens you collect in a museum collection. Select an institution that can provide long-term care for the collection and make it available to the scientific community. If you are collecting outside of the United States, you will probably be required to deposit a percentage of your specimens in a collection in that country.
3. Obtain the necessary permits. This may include a collecting permit, permission to conduct research, an export permit, or other documents.
4. If any specimens you wish to collect are protected, obtain the special permission required (e.g., a CITES permit).
5. Obtain the necessary supplies and field gear.
6. Collect, preserve, and document the specimens carefully.
7. When the trip is over, file the required reports with the permit authorities.
8. Write thank-you letters to individuals who assisted you in obtaining permits and doing fieldwork.

Bringing Specimens into the United States from a Foreign Country

1. Obtain CITES export and import permits, if applicable.
2. Complete a 3-177 declaration form, and make a copy.
3. Enter the United,States through a US Fish and Wildlife Designated Port (if required), or obtain a Designated Port Exemption permit in advance.
4. On your customs declaration form, declare that you are importing wildlife.
5. File the 3-177 form with a US Fish & Wildlife agent (or US Customs agent if no Fish & Wildlife agent is available). If the collection does not include any protected species (e.g., CITES listed or Threatened and Endangered species), you may file the 3-177 with the appropriate US Fish & Wildlife regional office within 180 days of your trip.
6. Ask the agent to stamp the 3-177 form and give you a photocopy of the stamped form.
7. If necessary, file an amended 3-177 form to correct errors in identifications or specimen counts later.

Ethics and the Importance of Collecting

Considering the number of preserved amphibians and reptiles already in museum collections, why do we need to collect more? Making new collections is important for several reasons. There are many gaps in existing collections yet to be filled in. Collections need to be made to support future research, particularly research utilizing new investigative techniques. In many places, amphibian and reptile populations are on the verge of disappearing entirely, making the importance of collections from these areas critical. Preserved specimens provide an ecological and evolutionary "snapshot" of the environment—they confirm the occurrence of a species at a specific place at a particular moment in time. There are still many species that are unknown, and many that have undescribed larvae or juvenile forms.

Specimens need to be collected as vouchers for field research and as vouchers for molecular samples. The appropriate sample size depends on the research being done. An adequate sample of specimens from one locality for most systematic herpetological work is generally considered to be at least 20 specimens per species, plus a range of available developmental stages. Dietary studies require much larger samples (Kovács and Török 1997).

Specimens should be collected with a purpose, not merely to add numbers to a collection. Take care to carefully prepare and document each specimen collected to maximize its usefulness for research and voucher purposes. Avoid taking samples that are needlessly large, particularly of well known, common species, but remember that samples that are too small are of little use. Many permitting agencies impose arbitrary and unscientific limitations on sample sizes. When this happens, try to convince the permit granting authorities of the need to collect adequate samples.

Under normal circumstances, it is highly unlikely that scientific collecting will damage a wild population. The mortality rate for most wild living amphibians and reptiles is very high. The number removed for an adequate scientific sample should have little effect on a wild population, unless that population is already perilously low in size and restricted to a small, isolated geographic area.

Euthanize specimens in a humane manner, while still rendering the specimen fit for good preservation. Record all data possible for each individual specimen that is collected.

Comply with all laws and regulations in effect in the area where collecting is done. Intentional violations of the law will result in serious problems for the collector, for the museum in which the specimens are later deposited, and reflect badly on the profession as a whole. For further information on collecting ethics, refer to Anonymous (1987), Association of Systematics Collections (1993), Herrera-MacBryde (1986), Loftin (1992), and Pearson (1986).

What to Take in the Field

Each field trip has its own supply and equipment requirements. For guidance in selecting what you will need, refer to *Appendix II*.

MAKING THE COLLECTION

> Bad work in collecting is, in nine cases out of ten, due to one of two causes—ignorance or laziness. By some curious process of reasoning, many really intelligent men conclude that they can go into the field and collect successfully without having learned a single thing about methods, or asked a word of advice from a competent instructor.
> —W.T. Hornaday (1905)

When to Collect

When to collect is dictated by habitat type or scheduling restrictions with regard to day vs. night and wet vs. dry seasons, but collecting at the "wrong" times can often yield animals otherwise overlooked. Don't let preconceptions about an area to be worked limit the choice of equipment and field clothing before departure for the trip. Go prepared to work day and night, rain and shine.

In general, for seasonal conditions, wet-season collecting is more profitable than dry-season collecting, although in many areas the dry season has the effect of concentrating wildlife around dwindling water resources, thus increasing the chances of obtaining some species. In some tropical regions, a few species breed largely during the dry season.

What to Collect

Most field collectors concentrate on adult animals. In fact, juveniles and particularly neonates of most amphibian and reptile species are rare in collections (Alberch 1985). Efforts should be made to collect all life stages of the species desired, including eggs and larvae, neonates and immatures, and a balance of both sexes in adults. Larvae should be raised in the field, if possible, with one or more individuals preserved at each stage to yield a developmental series (see *Eggs*). A small effort in the field will result in very valuable collections for future research.

When preparing for a collecting trip, take the time to carefully examine museum records and any specimens already in collections from the area to which you will be going. Collecting animals that are not needed can be avoided by becoming familiar with existing material.

How to Collect

Once you have donned appropriate field clothing (see *Appendix II*), equip yourself with:

- a variety of plastic bags
- several cloth bags
- a notepad and pen
- a series of sequentially numbered tags to drop in the collecting bags along with the specimens to help keep your notes straight (these are not the same as your field catalog tags)
- a hat
- a thermometer for air and water temperature
- a snake hook, tongs, or placenta forceps with serrated ring jaws
- a large knife or machete
- compass or GPS unit
- at night, a headlamp, extra bulb, and extra battery sets

As you collect animals, drop a numbered tag in the collecting bag with the specimen. Use this number to record collection data, time, temperature, and other pertinent information on your note pad. This information will later be rewritten in the proper format in your field notes. Keep bags of specimens out of direct sunlight. Put a few damp leaves or some moss in the bags with the specimens.

How Much to Collect

The limitations on how much to collect include those imposed by permit restrictions, ethical considerations, adequate sample sizes, and specimen preparation time. How many specimens to collect must be a balance of these often-conflicting factors. If several collectors are working in an area, it is very easy for the tide of incoming specimens to overwhelm the preserving and cataloging process (you can tell this has happened when the expedition leader, eyes studded red by formaldehyde fumes, staggers from the processing area and confiscates all the plastic bags in camp).

How Much Time does it take to Prepare Specimens?

As a general rule, for standard preparations, including note taking and specimen preservation, calculate an average of 10 to 20 minutes per specimen. Add another 10 to 20 minutes per specimen for tissue preparation, and an additional 15 to 20 minutes to photograph a specimen. Preparation time speeds up when preparing many specimens of the same species, and slows down when preparing fewer individuals of a larger variety of species. Add to this calculation approximately 30 minutes to set up the preparation lab, and another 30 minutes to clean up and break the lab down when preparation is completed. Finally, add at least 45 to 60 minutes per day for writing your field journal.

Places to Collect

Collecting may be associated with a particular project, such as obtaining voucher specimens for an ecological study, or specimens selected to monitor reproductive activity of organisms in a community. For this type of collecting, the locality is dictated by the requirements of the study.

For biodiversity surveys, when a sample of all species in an area is desired, collecting should be approached with an open mind. By day, turn over rocks, logs, and pieces of metal or wood lying on the ground. Walk paths and trails slowly to spot animals that are actively hunting or sunning themselves. Peel bark from dead trees, and rake through leaf litter. Use traps, and drift fences, or shelters if you are in an area for a reasonable length of time. By night, walk paths and trails with a light. Search the margins of ponds, streams, and swamps (try to familiarize yourself with the area you plan to collect while it is still daylight). Many good aquatic sites can be located at night by tracking calling frogs. Cruise roads slowly in a vehicle with headlights on low beam.

Many animals that are difficult to collect during the day, such as some species of lizards, may be plucked easily from their perches at night (Hoffmeister 1951).

Most collectors are familiar with the "edge effect" phenomenon—animals are often more plentiful at the edge of disturbed situations than they are in pristine habitat. Hunting along roads, fence rows, around human habitations, trash dumps, wells, irrigation ditches, and the like is often quite productive. In urban areas, check parks, trash piles, and even inside sunken utility meter boxes (McCoy 1961). Alert people to your activities and collecting needs. Local people, particularly children, often know exactly where to find the animals you need.

Following are some typical situations where reptiles and amphibians may be found.

Areas being Burned or Cleared. Many forms of wildlife flee encroaching fires. Watch for movement just ahead of the heat and flames, while keeping a cautious eye on the fire itself. Following bulldozers as land is cleared will often yield species otherwise difficult to find.

Caves. Very few amphibians and reptiles are found in caves, other than those that live near the cave mouth, although certain species of salamanders are found only in caves (Cooper and Poulson 1968).

Cavities. Holes in trees and stream banks and water collected in the bases of agaves or bromeliads and other plants are all excellent places to search for small reptiles and amphibians. Eggs or larvae are sometimes found in cavities in bamboo, hollow trees, or epiphytic plants. I once saw a *Leptotyphlops* crawl out of a hole in a pinecone when someone kicked it.

Deserts. By night, drive slowly along roads (see *Road Cruising*) and look around rocky areas with a light. By day, flip rocks and debris on the ground, search the cracks of exfoliating boulders or exposed shale, and the gaps between rocks (Morrison and Tanzer 1966). Spring rains in the desert (when creatures run riot in temporary pools) are the most productive times to collect, and may be the only time you will find most desert dwelling amphibians. Desert collecting may be improved by trapping (Banta 1957; see *Traps*). On fine-textured soils, snakes and lizards may be found by following their tracks (Bider 1968, Lillywhite 1982, Mosauer 1933). See also Whitlock (1971).

Hillsides and Pastures. Flip any shelters and covers, such as rocks, logs, and pieces of wood and metal. This is usually more successful on slopes of 45 degrees or less, particularly when the slope is oriented toward the sun. Thinner, flatter rocks generally are preferred over thicker ones. Be alert for eggs as well as moving creatures beneath the rocks. Always returned the flipped covers to a close semblance of their original position. If long-term work is planned in an area with open hillsides or pastures, shelters or covers made of wood or metal may be set out to attract amphibians and reptiles (see *Shelters and Covers*).

Leaf Litter. Sift systematically through leaf litter with a small rake, potato rake, snake hook, or machete by day. Keep an eye out for amphibian and reptile eggs. In some tropical regions, diurnal leaf-litter frogs can be located by listening for calls.

Oceans. Sea snakes may occasionally be caught in nets in the open ocean, found in tide pools, or be washed up on the shore. A few species come ashore intentionally. Sea turtles are notoriously difficult to collect except when ashore, unless they are accidentally taken in fishing nets. Both Humboldt (1896) and De Sola (1932) described how to collect sea turtles by hauling them in on a line tied to a remora or sharksucker (Echeneidae).

Ponds and Lakes. Shorelines should be worked with nets (see *Nets, Seines, and Netting*) by day and searched with a headlamp (see *Headlamps and Spotlights*) by night. Be alert for animals in the water and those coming down to the water's edge to eat the animals in the water. Traps for amphibian larvae and adult reptiles and amphibians are recommended (see *Traps*). Check man-made structures at ponds and lakes (Hawken 1951). Large frogs may be taken on lures or flies (Miller 1972). A plunge tray may be used to collect organisms in the water (Karns 1986).

Road Cruising. This classic technique for snake hunting in the Age of Automobiles is very effective (Klauber 1935, O'Shea 1992, Shaffer and Jutterbock 1994). If seldom traveled roads are available, particularly asphalt, cruising at slow speeds beginning at dusk is a good way to find specimens that crawl out on the road for warmth. Be prepared to leap from the vehicle quickly, and take a flashlight with you. Exercise care when road collecting to avoid traffic, particularly in places where approaching traffic may not be clearly visible. A spotlight may be used to search for creatures on the sides of road cuts. A variety of powerful lighting devices that operate using a car battery are available, but note that their use is illegal in some jurisdictions. Road cruising itself is illegal in some areas, so check local regulations first. A scoop device has been described for rapidly retrieving animals off the road (Sievert et al. 1999).

Roadside Ditches and Temporary Pools. These ephemeral habitats often harbor amphibians and reptiles, particularly immediately after a rainfall. Many amphibians breed only in ephemeral pools.

Streams and Rivers. Night collect streams and rivers by walking along the bank or in the water while wearing a headlamp (see *Headlamps and Spotlights*). Check exposed rocks and overhanging vegetation as well as stream banks and in the water. By day, roll rocks in the streambed, use dip nets or seines, and set out aquatic traps (see *Traps*). Animals may hide in cavities created by streams that undercut their banks (see *Noodling*). Amphibian larvae blend in well with stream bottoms and hide among the gravel, leaves, and detritus. Some have sucker mouth parts that allow them to clasp the undersides of rocks, and can be caught by a downstream net when rocks are moved (Svihla 1959).

Streetlights, Porch Lights, Building Lights. Outdoor lighting attracts many insects, which in turn attract a variety of amphibians and reptiles.

Swamps. Bogs, swamps, and seepages are best collected for most species at night with a headlamp (see *Headlamps and Spotlights*), or worked by day with nets. Swamps are excellent places for aquatic traps for amphibian larvae, turtles, and other animals (see *Traps*).

Termite Mounds and Ant Nests. Snakes, amphisbaenians, and some amphibians may be found in the soft earth around the base of termite mounds. It is easier to dig them out if you can first tip the termite mounds with a tractor or with a lever. Many tropical ant species construct large ground nests of loose material that harbor small snakes and frogs.

Tree Falls. Both natural tree falls and those resulting from human activity often yield seldom-collected species. Check carefully in the roots of the fallen tree as well as on the trunk, under the bark, and in the upper branches. Particularly large fallen tropical trees may have buttresses that fill with water and form breeding sites for amphibians.

Tree Tops. For the adventurous or the well insured, the canopy of the forest can be accessed using modified rock-climbing techniques and lots of rope. Canopy level bromeliads and other epiphytic plants are particularly good places to look for amphibians and reptiles. A technique has even been developed for using drift fences in trees (Vogt 1987). For the thrilling details of collecting in the treetops, refer to Mitchell (1982), North (1943), O'Shea (1992), Perry (1978), Tucker and Powell (1991), and Whitacre (1981).

Tropical Forests. By day, roll logs, peel bark, and sort through leaf litter. Myers (1956) described a method of catching small lizards on the forest floor by dangling an insect tied to a thin line on the end of a pole in front of likely looking spots among leaf litter and debris. Bushes may be beaten for snakes and lizards. By night, walk trails and paths using a light source (see *Headlamps and Spotlights*). Tropical forest vegetation can be quite overwhelming at first, and it takes time to form a search image of your intended prey. Give yourself time to adapt to the conditions. Remember to look up into the trees and down at the forest floor as you move along trails, and not just search at comfortable eye level. Steep-sided trenches may be dug or pitfall traps placed when the collector will be in residence for a lengthy period of time (see *Traps* and *Trenches*). Always cave in or fill in trenches and holes from pitfall traps when collecting in the area is completed.

Collecting Techniques and Equipment

When traveling to remote areas or out of the country to collect, never plan on purchasing any supplies locally unless you know you will be passing through a large city and have plenty of time on your hands. The simplest items can be the hardest to locate (see *Appendix II* for more information on what to take with you, and *Appendix III* for sources of supplies and equipment).

Bags. Plastic bags are recommended for amphibians and many small reptiles. You can never have too many with you. Buy two mil (0.002 inch) plastic bags with long necks that can be easily tied shut. I prefer 6 x 15 inch and 8 x 18 inch bags. Avoid zipper bags (e.g., Zip-Loc®) and wire clamp bags (e.g., Whirl-Pac®), as it is difficult to get sufficient air in these bags for the animals to breathe, the bags are not easy to carry once the animal is inside, and the air may escape easily from them. I recommend wearing a belt while collecting so that bags may be tucked under the belt for safe keeping, leaving both hands free, or putting the plastic bags in a cloth bag that is tied to the belt. Put a bit of damp leaf litter or moss inside the bag for moisture. Plastic bags may be reused several times if washed thoroughly (with water—no soap) unless they have had a particularly noxious animal inside (e.g., certain amphibians with highly toxic skin secretions). Cloth bags should be made of double washed (to remove the sizing) white cotton or muslin, with double-sewn seams, and be deep enough to be safely tied with a venomous snake inside (see Huheey 1964). I recommend cloth bags of approximately 12 by 24 inches and 18 by 32 inches. The proper technique for bagging a venomous snake is to (1) drop the snake in the bag, (2) quickly lay the bag on a flat surface, (3) confine the snake in the bottom of the bag by holding the shaft of a snake hook across the bag, and (4) tie the top of the bag tightly.

Bait. Some species of amphibians and reptiles may be attracted to areas where they are easy to snatch by using food bait to attract the insects that they feed on or to attract the animals themselves (Wilson and Pine 1974). Insects or fruits may be tied by a thin thread or monofilament line to a pole and dangled before an amphibian or lizard. Grab the beast when it grabs the bait (Myers 1956, Serena 1980). Small hooks baited with live insects may be dropped down the burrows of salamanders (Mount and Schwaner 1970). Bait may also be used with adhesive traps (see *Traps*).

Blowguns. These devices are recommended for diurnal collecting. Hoddenbach (1966), Karns (1986) and Tinkle and Lawrence (1956) describe how to make blowguns from a smooth tube using protruding needlepoint darts. A commercial blowgun (60 inches long, 40 caliber, made of Teflon® lined aluminum tubing) has also been recommended (L. Grismer, pers. comm.). The commercial blowgun fires plastic stunplugs (see *Appendix III*).

Water Pistol. According to Myers (1956), small lizards can be dislodged from walls and trees with a water pistol (the "E.R. Dunn method..."). The newer pump action hydraulic water pistols (e.g., SuperSoaker®) provide more force and a larger fluid reservoir.

DRIFT FENCES. Fences are used in conjunction with shelters (see *Shelters and Covers*), pitfall traps, trenches, and funnel traps (see *Traps*). Drift fences are useful devices when a collector is in an area for an extended stay. Traps and trenches must be checked regularly, several times each day. Drift fences may be made of wire mesh, metal, cloth, plastic, or wood.
General. Campbell and Christman (1982), Enge (1997), Gibbons and Semlitsch (1982), Karns (1986), Middendorf (1979), O'Shea (1992), Smith (1971), Vogt and Hine (1982).
Aquatic Drift Fences. Lutterschmidt and Schaefer (1996).
Arboreal Drift Fences. Vogt (1987).
Drift Fences for Amphibians. Arntzen et al. (1995), Corn (1994), Dodd (1991), Dodd and Scott (1994), Gibbons and Bennet (1974), Moulton (1954), Murphy (1993), Storm and Pimentel (1954), Sutton et al. (1999).
Drift Fences for Reptiles. Cockburn et al. (1979), Fitch (1951), Hall (1970), Milstead (1959).

Eggs. Reptile and amphibian eggs should be hatched under field conditions, if possible. Preserve reptile young immediately. Reptiles may be tricked into depositing their eggs in troughs filled with attractive nesting materials (Gordon and Tinkle 1960). Reptile eggs can be incubated in their native soil in a closed plastic bag or clean plastic container, opened occasionally for air; in a 1:1 mixture (by weight) of sterile potting soil (vermiculite) and distilled water (Tryon 1975); or in moist sterile cotton (Legler 1956). Amphibian eggs may be incubated in a plastic bag or clean plastic container, with water changes as necessary. An amplectant pair of amphibians may be kept in a clean plastic bag until eggs are deposited. Once the eggs hatch, try to raise the larvae through metamorphosis, preserving one or two at each developmental stage (for stages, refer to Duellman and Trueb 1986). Feed the developing amphibian larvae on pond detritus or tropical fish food (e.g., Tetramin Tabs®).

Elastic Bands. Big rubber bands are extremely useful for stunning small lizards or field companions long enough to grab them. Dundee has described a gunstock to fire elastic bands with greater force (Dundee 1950). See also Brown (1946) and Neill (1956). Elastic bands can also be used on frogs at night and on roaches in your hotel room.

Electrical Devices. Several electrical apparati to stun or shock reptiles and amphibians in the water or in the ground have been proposed. For details, see Anderson and Smith (1950), Gunning and Lewis (1957), Harris (1965), Joanen and Perry (1971), and Smith (1971).

Forceps. Long forceps (bottle forceps) are very useful for getting animals out of cracks and holes, and for handling preserved specimens. Purchase a pair 12 inches or longer. Scissor-grip placenta forceps with serrated ring jaws are also quite useful, particularly for small venomous snakes. See *Appendix III* for suppliers.

Gigging and Gaffing. A long pole with a sharp point or points may be used to collect a variety of amphibians and reptiles (Lagler 1943, Walden 1970). In some areas gigging is the only legal means to collect game species of frogs, however, it is not approved as a humane collecting technique (Anon. 1987).

GPS Device. A global positioning system (GPS) device that takes bearings from satellite transmissions is an indispensable field tool. Prices range from around $100 to $600. Desirable features include parallel channel tracking, fast signal acquisition time, and a clear, easily read display.

Grabbers. Several grabbing devices have been described for use with small, nimble animals. See Bedford et al. (1995), Durtsche (1996), and Witz (1996).

Headlamps and Spotlights. The use of eyeshine is indispensable when collecting at night. It is particularly useful in dense habitats, in road cuts, on cliff faces, and on walls. To see eyeshine, use a light source that can be positioned close to the level of your eyes, with focussing lens. There are a variety of battery-powered headlamps on the market. In general, larger batteries (e.g., six-volt) provide brighter, longer lasting light than do smaller batteries (e.g., AA size). What size headlamp to use is a trade-off between weight, availability, and cost. I prefer a six-volt headlamp that uses four D-cell (1.5 volt) alkaline batteries. Mark the batteries and switch battery sets every hour to prolong battery life. Setting batteries out in the sunlight will help recharge them. See also Corben and Fellers (2001), O'Shea (1992), and Whitaker (1967b).

Jars and Vials. Small plastic jars and vials with snap-on or screw-on lids make handy containers for small reptiles and amphibians, and prevent the animal from being crushed as can happen with plastic bags. Jars and vials can be reused if washed thoroughly with water.

Local Residents. If you will be in one place for a reasonable length of time, it is often profitable to offer monetary remuneration for specimens. Resident children are often better local collectors than the visiting field person is. Herpetologists have even gone so far as to broadcast their desires for specimens over the local radio station (Shaw 1962). Careful questioning will be necessary to obtain the requisite information concerning the location and habitat of the individual specimen when someone else collects it. Price adjustments must be made over time to discourage over-collecting of common species. Other local life forms may also aid in the location of desirable specimens, as Smith (1946) discovered with flocks of snake-finding West Texas turkeys.

Machete. Very useful in thick vegetation for cutting trails, poles, and body parts. Machetes can also be used for digging holes and raking through leaf litter. Purchase a machete with a sturdy scabbard that can be worn on your belt. Take along a good file to keep an edge on the machete blade.

Nets, Seines, and Netting. Dip nets (of various sizes) and tea strainers are useful for collecting amphibian larvae. Also useful are dredges and seines (Goin 1942). Meylan (1989) recommended a 6-10 meter bag seine. Bragg (1951) described a straining device for collecting aquatic organisms using a wooden frame and screen. Nets for collecting non-aquatic species have also been described for lizards (Bouskila 1985, Paterson (1998) and turtles (Lagler 1943, Recht 1981), and Vogt (1980).

Noodling. This ancient technique involves reaching under water with bare hands to grope around for an animal, then grabbing the beast (Karns 1986, Lagler 1943, Plummer 1979). It is a particularly useful technique for working undercut stream banks. Walking barefoot in the mud of ponds and swamps has also been used in the Venezuelan llanos to locate both caiman and anacondas (S. Manness, pers. comm.).

Noosing. This standard technique is used for collecting diurnal lizards, but it may be easily adapted to other types of amphibians and reptiles (Karns 1986). Use a slipknot of monofilament, thin thread, or dental floss and a long, thin pole. Work the noose slowly over the animal's head, then jerk upwards with a quick motion. Variations include dangling bait or a rubber band lure in front of an animal or in front of its lair to lure it to the noose (Peaker and Peaker 1967). Strong et al. (1993) described a baited noose. The noose may be stiffened by waxing the line or may be made of copper wire (Eaken 1957). Nooses may also be shot from a noosing gun (Bertram and Cogger 1971), used with snap snares (Stickel 1944, Bennett et al. 2001), or noose traps (Bennett et al. 2001, Vyas1990, and Ziegler 1999). Other descriptions include using nooses to get animals from crevices (Morrison and Tanzer 1966), noosing salamanders (Camp and Lovell 1989), and skinks (Durden et al. (1995).

Pistol. Regulations make use of a firearm extremely complicated in most places, but a .22 caliber pistol with a long barrel (6-12 inches), loaded with dust shot is useful for collecting fast-moving, diurnal lizards which are difficult to noose (Schmidt 1951). Air pistols firing BBs or pellets are also very effective.

Poles. These are useful for several purposes. For diurnal lizard collecting, you can use them for noosing (see *Noosing*). By night, many animals, such as frogs, can be coaxed onto the end of a pole and then lowered from the upper reaches of the vegetation to the waiting hand of the eager collector. A long pole can be used to knock an animal from a high perch. A stout pole can be used as a walking stick in steep terrain, in mud and muck, and to help the collector move in swift stream currents. Carpenter (1955) located turtles in piles of leaves and debris by gently probing with a thin pole. Collapsible, telescoping poles are available, and of course in wooded areas one can always be cut on the spot.

Rakes. A small rake, particularly a potato rake, is useful for combing through leaf litter, flipping objects, going through aquatic debris, and locating aquatic turtles buried in soft sand under shallow water (Meylan 1989).

Scoops. These are used to collect animals from roadways, leaf litter, and other situations (Brattstrom 1996, Sievert et al. 1999).

Shelters and Covers. Shelters and covers are objects (such as pieces of wood, metal, or plastic pipe sections) that are positioned for animals to hide under or inside. A pitfall trap may be used beneath a shelter. Examples of how a temporary shelter may be used include tossing a hat on the ground and allowing lizards to run under it (Klein 1951); placing cardboard tubes (with one end closed) on the ground (Strong et al. 1993); or setting out PVC pipe tubes for frogs (Moulton et al. 1996). For more information on shelters and covers, refer to DeGraaf and Yamasaki (1992), Fellers and Drost (1994), Fitch (1992), Grant et al. (1992), Parmelee and Fitch (1995), Strong et al. (1993), and Sutton et al. (1999).

Shovel. A shovel is a heavy item that can be hard to pack, but it can be very useful for digging trenches, pitfall traps, and extricating field vehicles from tactical miscalculations. The legendary folding entrenchment tool is a good compromise, especially if you have an assistant to do the digging with it.

Slingshots and Bean Shooters. These have been suggested as alternatives to a pistol. Slingshots take some skill to use and a ready supply of ammunition (Engelhardt 1917).

Snake Handling Devices. A large number of variations on the theme of hooks, nooses, clamps, tongs, rakes, and buckets have been developed for handling snakes. Do not use a collapsible hook when handling venomous snakes. Several manufacturers make short tongs and hooks that are easy to pack and carry in the field (see *Appendix III*). Among snake handling devices proposed are containers (Freed and Freed 1983, Gillingham et al. 1983); hooks and rakes (Glusenkamp 1995); injecting sticks (Jennings and Vindum 1991); strap and restraining sticks (Gregory et al. 1989, McDonald 1964, Ward and Harrell 1978); tongs (Pillstrom 1954); tubes (King and Duvall 1984, Murphy 1971, Rivas et al. 1995, Sutherland and Hampton 1961, Walczak 1991); and wire mesh cable holders (Mauldin and Engeman 1999). Other general references to snake handling devices include Almandarz (1986), and Altimari (1998).

TRAPS. There are many types of traps and trapping techniques for amphibians and reptiles. Traps must be checked frequently, throughout the day and night, or disabled so that they will not continue to trap animals. Following is a list of references to traps by type of trap.

Adhesive Traps (Sticky Traps). These are usually glue boards intended for catching rodents or insects. Custom shapes and sizes may be made by applying bird Tanglefoot® to a sturdy cardboard backing. Live animals may be safely removed from the adhesive by using a diluted vegetable oil or mineral oil solution. Adhesive traps must be monitored closely and checked frequently. It is advisable to anchor them securely to a fixed object. Remove animals from the traps immediately by gently working a small amount of the diluted oil solution under the animal with your fingers. For more details, refer to Bauer and Sadlier (1992), Downes and Borges (1998), Durtsche (1996), Glor et al. (2000), Rodda et al. (1993), Vargas et al. (2000), Whiting (1998), Whiting and Alexander (2001), and Zani and Vitt (1995).

Funnel Traps. These traps may be made from wire mesh, netting, or metal. A funnel trap consists of a cage or other container with a funnel-shaped entrance (see Figure 1). The funnel opening makes it difficult for an animal to escape the trap once it has entered.

Figure 1. Funnel Trap

Aquatic Funnel Traps: A simple funnel trap for aquatic larvae can be made out of an empty two-liter plastic bottle by cutting off the top and inserting it backwards into the bottle. In many areas, indigenous hunters weave aquatic funnel traps from plant fibers. General descriptions (Adams et al.1998, Richter 1995); trap construction (Graham and Georges 1996), surface aquatic funnels (Casazza et al. 2000), salamanders (Carpenter 1953, Griffiths 1985), amphibian larvae (Smith and Rettig 1996), snakes (Fraker 1970), turtles (Feuer 1980, Iverson 1979, Legler 1960, Tucker (1994), use of decoys for turtles (Mansfield et al. 1998).

Arboreal Funnel Traps. Fritts et al. (1989), Rodda and Fritts (1992), Savidge (1987), Vogt (1987), and Zani and Vitt (1995).

Terrestrial Funnel Traps. Clark (1966), Dargan and Stickel (1949), Doan (1997), Enge (1997), Fitch (1951, 1987), Greenberg et al. (1994), Imler (1945), Karns (1986), Keck (1994), Lohoefener and Wolf (1984), Mansfield et al. (1998), Mushet et al. (1997), Vogt and Hine (1982), and Zani and Vitt (1995).

Bai-chatri Traps. These are traps for basking turtles, made by attaching monofilament slip nooses to a section of poultry mesh fencing (Braid 1974). The fencing is then molded around a basking log or other basking site.

Live Traps: Mammal-style live traps may be used for larger lizards, or the traps may be modified to accommodate a particular lizard species (Doan 1997, Durden et al. 1995, Heatwole et al. 1964, King et al. 1994).

Pitfall Traps and Trenches. The simplest form of pitfall traps is merely a hole dug in the ground. I have seen these used for turtles, baited with a piece of fruit stuck on the end of a stick protruding from the bottom of the trap. The use of poisons or other killing agents in pitfall traps is not recommended for two reasons—most of the available chemicals are inhumane, and the interval between death and fixation is likely to be unacceptably long. For general references on the use of pitfall traps, see Corn (1994), Karns (1986), and O'Shea (1992). Evaluations of pitfall traps are provided by Banta (1957), Greenberg et al. (1994), Sutton et al. (1999), and Vogt and Hine (1982). Flip-top traps are described by Christiansen and Vandewalle (2000) and Nadorozny and Barr (1997). Crawford and Kurta (2000) evaluated the color of traps, Daoust (1991) discussed ways to avoid the dehydration of trapped animals, and Whitaker (1967a) discussed using bait in pitfall traps.

Sheet Traps. This technique involves spreading a plastic or cloth sheet on the ground, constructing an artificial habitat on it to lure the unsuspecting animal, then lifting it off the ground for capture (Allan et al. 2000, Mauldin and Engeman 1999).

Shelter Traps. An artificial shelter can also be used to attract and trap animals. Moulton et al. (1996) described using PVC pipe sections, Strong et al. (1993) suggested plastic or cardboard tubes; Parker (1971) discussed the influence of trap covers.

Miscellaneous. Bryan et al. (1991) described a spring loaded turtle trap, Heatwole et al. (1964) used baited snap traps and mammal live traps, Lannon (1962) suspended a dry fly over a pitfall trap, and Vogt (1941) described a shelter and pitfall trap.

Specialized Techniques for Particular Organisms

Anurans. Collecting bullfrogs by hand (Murphy 1968); locating *Heleophryne* eggs (Visser 1971).

Caecilians. Digging in damp areas is said to be a good way to turn up caecilians. The late Edward H. Taylor, when asked his secret for using this method so successfully, offered me this advice: "Don't waste your time digging where you are not going to find anything." Caecilians may be found in tropical forests, rice paddies, coffee and tea plantations, streams and rivers (Hofer 2000).

Salamanders. Newt trapping (Boarder 1969); *Cryptobranchus* collecting (Soule and Lindberg 1994, Williams et al. 1981); plethodontid collecting (Valentine 1963).

Crocodilians. General capture techniques (Chabreck 1963; Jones 1965, Murphy and Fendley 1975); use of electricity (Joanen and Perry 1971); rope nets and trap boards (Webb and Messel 1977); snap snares (Mazzotti and Brandt 1988).

Lizards. Oatmeal bait (Jorgensen and Orton 1961); under bridges (Conant 1951); trap-door box for teiids (Rodgers 1939); noose trap for arboreal lizards (Bennett et al. 2001); spotlighting geckos (Whitaker 1967b); sand skinks (Rathor 1970).

Snakes. Locating snake dens (Jackley 1947); trapping water snakes (Franklin 1947); *Charina* (La Rivers 1974).

Turtles. Bait (Ernst 1965); basking traps (MacCulloch 1978, Petokas and Alexander 1979); decoys (Mansfield et al. 1998); restraint (Galbraith and Brooks 1983); sounding (Carpenter 1955); finding turtles in winter (Bishop and Schoonmacher 1921); freshwater turtles (Bider and Hoek 1971, Iverson 1979, Iverson 1991, Lagler 1943, Vogt 1980, Webb 1961); *Gopherus* trapping (Bryan et al. 1991); *Graptemys* (Chaney and Smith 1950, Wahlquist 1970); softshell turtles (Robinson and Murphy 1975).

Evaluations of Capture Techniques.

General techniques: Mitchell et al. (1993), Parris et al. (1999), Sutton et al. (1999). ***Drift fences, funnel traps, shelters, and pitfall traps***: Arntzen et al. (1995), Brathwaite (1983), Bury and Corn (1987), Campbell and Christman (1982), Christiansen and Vandewalle (2000), Enge (2001), Friend et al. (1989), Greenberg et al. (1994), Hobbs and James (1999), Lohoefener and Wolfe (1984), Morton et al. (1988), Parmelee and Fitch (1995), Smith and Rettig (1996). ***Collecting amphibians***: anurans (Brown 1997), newts (Cooke 1995), Fronzuto and Verrell (2000), Griffiths and Raper (1994). ***Collecting lizards***: *Phrynosoma* (Fair and Henke 1997). ***Collecting snakes***: Fitch (1992). ***Collecting turtles***: Frazer et al. (1990).

PHOTOGRAPHY

There are several good books on field photography that provide a wealth of useful hints for taking good pictures, particularly those by Baker (1976), Moldvay (1981), Shaw (1987) and West and Leonard (1997).

Camera. Select a sturdy 35-mm or digital camera that will accept a lens that enables you to take close-up photographs of small reptiles and amphibians. Get a sturdy carrying case and camera bag. Always carry extra camera batteries.

Color Film. Historically, most field workers preferred to shoot color transparencies, because a good print could be made from a slide, but not vice versa. However, a good print can now be digitized and used as an electronic image or reformatted as a transparency. For photographing animals, select a film with an ISO rating of 100 to 200 for the best compromise between speed (amount of light needed) and clarity of image.

Lenses. Although a 50-mm lens is the standard for 35-mm cameras, to avoid lugging around a lot of photographic equipment, I recommend a short zoom-macro (30-80 mm is a popular choice), which can be used for everything from habitat shots to close-ups. A good quality macro lens produces the best clarity of image for close-ups, but these lenses are expensive. Macro ratios vary from 8:1 down to 1:1. The closer to the 1:1 ratio, the better, as long as you can maintain an f-stop of f-16 or higher to provide an adequate depth of field. Approximate cost of close-up apparatus is listed in Table 2.

Table 2. Approximate costs of close-up photographic equipment.

Type of lens or attachment	Price range in US $	Quality of close-up image
Diopter lens set	30-100	Very good
Teleconverter (2x)	50-300	Good to very good
Bellows	100-200	Good, but poor depth of field
Extension rings (set)	50-150	Good, but poor depth of field, lacks the variable range of bellows
Zoom-macro lens	80-300	Good to very good depending on the lens quality and macro ratio
Macro lens	225-500	Excellent, best depth of field

No matter what lens you use, always keep a filter on the lens to protect it. I recommend a Tiffon 812® color-enhancing filter, but skylight filters are also popular for this purpose.

Electronic Flash. Use a "dedicated flash" that is metered through your camera. Most flash units do not function automatically for close-up work, so allow yourself the time and necessary rolls of film to experiment with close-up exposures until you are satisfied with the results. The use of an electronic flash makes a big difference in the quality of a photograph, whether it is used as the principal light source or for fill-in lighting. Always take extra flash batteries in the field.

Lens Cleaning Cloth. Purchase a lens cleaning cloth or lens cleaning kit that is small and easy to carry in a camera bag.

Photographing Specimens in the Field

Before you go in the field, shoot several rolls with your lens, camera, and lighting setup to determine the proper exposure settings. Take close-ups and habitat practice shots before you begin fieldwork. In the field is no place to try to figure things out. Become very familiar with your equipment.

When photographing an animal, pose it on a natural looking background. Reptiles and amphibians usually have to be placed in position several times before they will remain still long enough to be photographed. Be patient. One trick that will save you a lot of frustration when posing animals is to position the specimen where you want it on the background, then cover it with an object or your hand (Twiest 1968). Compose your shot, remove the covering, and snap the picture. Specimens may also be placed in a bucket of water and allowed to swim for a time. This tires small animals so that they can be more easily posed (J. Caldwell, pers. comm.). Photograph the venter of specimens as well as the dorsum, if possible.

Some photographers prefer to chill specimens in a refrigerator or ice chest before posing them, but this technique may alter the colors of the animal. Kaiser and Green (2001) have suggested rubbing the venter of small animals with a small amount of benzocaine (ethyl p-aminobenzoate) based oral analgesic (e.g., Oragel®) as an anesthetic. This should be done with caution, as the same chemical is also used to euthanize animals. See Mobbs (1978) and Twiest (1972) for other hints.

Carefully record each photograph you shoot, using a roll number and frame number system. It is particularly important to record the field catalog number of each individual specimen photographed. See Figure 2 for a suggested photographic record format.

Figure 2. Standard format for photographic record.

PHOTOGRAPHIC RECORD

Locality ______________________________

Roll Number _____ Film Type _______ Camera ______________

No.	Date	Subject	Field #	Lens	f-stop	Flash
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						

SOUND RECORDINGS

The first known recording of an animal sound was a bird, recorded in 1889 (Boswall 1969), but recording of frog calls is still ignored by most field herpetologists. With modern lightweight recorders, it has become much easier to make quality recordings with high research value. Differences in vocalizations are an important way to distinguish between some closely related species of frogs.

Recorder. Portable field recorders come in two formats, analog and digital. Analog recorders include reel-to-reel and cassette. Digital recorders are capable of capturing a cleaner sound sample than tape recorders, but they are not as rugged for field use and may have serious memory limitations. Reel-to-reel is superior to cassette tape because the tape surface is much larger and the recording speeds are faster. The larger surface area and faster speed mean that more magnetic particles are magnetized, resulting in a higher quality recording. Cassette recorders are smaller and easier to use in the field. The durability of the recording machine and the microphone, particularly resistance to dust, high relative humidity, and shock, is a prime consideration for field use. In general, these are the characteristics to look for:

1. Width of the recording head (for tape recorders)
2. High speed recording capability (for tape recorders).
3. Frequency range of both recorder and microphone.
4. Recording level meter with a wide dynamic range.
5. Durability of the recorder and microphone.
6. Recording monitoring system (speaker or headphones) with playback capability (speaker) to stimulate frogs to call.
7. Good, sturdy carrying case and shoulder strap.
8. Counter (to help locate particular portions of the recorded sound for playback).
9. Type of battery—select a model that accepts widely available batteries.
10. Standard microphone and accessory jacks.
11. Output jack to plug into a sound analyzer system back in the laboratory.

Microphone. It is the microphone that determines what your recorder will have available to record. Select a microphone with wide dynamic range. Unlike recording birds, most frogs can be approached very closely without having to resort to a parabolic reflector, although these and shotgun microphones can be very useful in the field. See Sinderson (1975) for details on how to make your own parabolic reflector.

Tape. All tape is not equal. Thinner tape means more will fit on a reel or in a cassette, but it is easier to break and more susceptible to noise "print-through" (unwanted echoes of sound that bleed through the tape). Use tape of at least one mil thickness, with low noise, high bias, and low distortion characteristics.

Field Recording Techniques

Before you head off to the wilds, don't just know how to operate your recording equipment, know how to operate it in the dark. Sinking crotch deep into a leech-infested swamp with the recorder hanging around your neck and the batteries in your headlamp giving out is no time to discover that you can't remember which button is *forward* and which is *rewind.*

Have the following ready when you want to record frog songs:
1. Notepad to jot down data which you might accidentally erase off the recording, and to help you keep your recorded segment numbers straight.
2. A series of sequentially numbered water-resistant tags to drop into the plastic bag with the individual specimens you record and collect.
3. Enough plastic bags to keep each recorded individual separately.
4. Thermometer to determine air and water temperature. This information may be crucial for the later interpretation of the recording. If possible, use a digital device that provides information on both temperature and relative humidity of the air. Be sure to position the device away from your body when taking measurements.

Before you began recording an individual frog, make a recording of the entire chorus of noises at the site for a few minutes. This will be useful later to help identify background noises and to filter out extraneous noise. This segment may also be useful to encourage the frogs to call again once you have entered the thick of the chorus to find it suddenly gone silent (Legler 1964). Whether recording a chorus or an individual, follow these basic steps:
1. Position yourself in a place where you can stand still and get a clear recording of the desired sound.
2. Using the recording level meter and monitoring system, check to see that you are picking up the sound you want to record.
3. Give the individual frog time to begin behaving normally after you are in place to record. Recording diurnal frogs requires extra care and patience, as they are much more wary than nocturnal species. Locate the frog you wish to record, set up the equipment, then sit down and wait patiently for the frog to resume its calling site and began vocalizing.
4. Start the recording, holding the microphone as still as possible and shielded from the wind. Record the frog for at least five minutes without interruption. This length of recording is necessary for analysis of calls per minute, intervals between calls and encounter of reciprocal calls from other males.
5. When the desired sequence has been recorded, collect the individual you have recorded. Recordings are much more valuable when you can collect the calling frog as a voucher. Bag each recorded individual separately with a number in the bag.
6. Play the recording back to make sure you got what you intended to record. At the end of the segment, record the segment number, date, locality, time, air and water temperature, relative humidity, species recorded, and identifiable background noises (including other species of frogs).
7. If previously recorded calls are used to stimulate a chorus or individual to begin calling again after they have been disturbed (Legler 1964), note this in the tape log in your field book along with other information about the recording.

SNAKEBITE

Although snakebite is not a common occurrence among field workers, it does happen. The more often venomous snakes are handled, the greater the risk from snakebite. Always presume that a snake is venomous if you are not positively sure of what it is. Always handle snakes by the safest method available (e.g., use tongs or tubes instead of pinning the snake and picking it up by hand).

If snakebite does occur, check for signs of envenomation before beginning treatments such as antivenin or surgery. It is estimated that at least 30-50% of venomous snakebites are dry bites, in which no envemomation occurs (Norris and Minton 1995). Be aware that elapid bites may not show symptoms for several hours after the bite.

Do not use ice, electric shock, tourniquets, "cut and suck" treatment methods, or topical agents to treat snakebite. These may increase the risk of tissue damage or death.

Viper bites. Signs and symptoms from viper bites may include pain, swelling, bloody ooze from the puncture marks, weakness, dizziness, nausea, vomiting, chills, sweating, odd taste sensations, headache, tingling of the skin, and muscle weakness.

- If you are within a few hours of medical care, get to a qualified physician as soon as possible.
- If you are more than a few hours away from medical care, a pressure bandage (not a tourniquet) may be applied, wrapping the entire bitten appendage. Apply the same pressure as for a sprained ankle.
- Although some people recommend the use an Extractor® snakebite kit (see *Appendix III*) immediately after the bite, there is evidence that this device may not remove a significant amount of venom and may cause more harm than good. Its use will also interfere with the application of a pressure bandage. In any case, do not make any cuts in the skin.
- Support the bitten area with a splint or sling.
- Transport the victim to medical help as soon as possible.
- The victim may be given fluids (if there is no nausea), but do not give beverages containing alcohol or caffeine.
- At the hospital, suggest that the attending physician start an intravenous (IV) line immediately, and consult with the regional poison control center.
- Before antivenin is administered, remind the physician to be prepared for anaphylaxis (an allergic reaction to the antivenin).

Elapid and Hydrophiid bites. Signs and symptoms from elapid and hydrophiid bites may include pain, numbness, muscle weakness, paralysis, difficulty breathing, difficulty swallowing, and difficulty speaking.

- Apply a pressure bandage (not a tourniquet) as quickly as possible, wrapping the entire bitten appendage. Apply at the same pressure as for a sprained ankle.
- Do not use an Extractor® or any other type of snakebite kit. Do not make any cuts in the skin.

- Support the bitten area with a splint or sling.
- Transport the victim to medical help as soon as possible.
- The victim may be given fluids (if there is no nausea), but do not give beverages containing alcohol or caffeine.
- At the hospital, suggest that the attending physician start an intravenous (IV) line immediately, and consult with the regional poison control center.
- Before antivenin is administered, remind the physician to be prepared for anaphylaxis (an allergic reaction to the antivenin).

PART III. PRESERVATION OF SPECIMENS

> Grave Digger: ...A tanner will last you nine year.
> Hamlet: Why he more than another?
> Grave Digger: Why, sir, his hide is so tanned with his trade that he will keep out water a great while, and your water is a sore decayer of your whoreson dead body.
> —William Shakespeare, *Hamlet*, Act V, Scene 1

Specimens should be processed as soon as possible after they are collected to ensure that the specimens are in the best possible condition. Some field workers prefer to preserve specimens immediately after capture, which usually means working by artificial light. Others wait until morning to preserve specimens collected at night in order to take advantage of natural light, although this postpones preservation and cuts into morning field time.

Field notes should include the time of capture, time of death, time of fixation, and a description of the precise methods used for killing and fixing specimens on each collecting excursion. Include the sources of chemicals used and how they were prepared in the field (e.g., mixed with stream water). This information will be important for future chemical analysis of the 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 (see *Conservation of Herpetological Collections*). Follow proper procedures carefully, and make sure that chemicals used for fixation and preservation are fresh and of proper strength.

THE FIELD KIT

A variety of field kits to carry the necessary items needed for collecting and preservation, may be found, ranging from a custom box (e.g., Maslin and Swenson 1971) to a small tackle box (J. Campbell, pers. comm.). Whatever the size and shape, be sure it contains the following items (see also *Appendix II*):

1. Hardening trays (polypropylene) with tight fitting lids (the first time you spend the night in a hotel room with a tray full of formaldehyde and a leaky lid, you'll know why...).
2. Paper towels to line the hardening tray.
3. Formaldehyde (full strength).
4. Packets of pre-mixed buffers for formaldehyde.
5. Small graduated cylinder to measure formaldehyde and water mixtures.
6. Containers for mixed 10% formalin solution.
7. Field tags, sequentially numbered, and with strings attached, ready for affixing to specimens.
8. Field book (ring binder) with 100% rag paper or spun-bonded polyethylene sheets.
9. A technical pen with a fine point.
10. A good supply of archivally stable ink (see *Field Notes*).

11. A number two pencil, pencil sharpener, and good quality eraser.
12. A few containers for handling live specimens during processing.
13. Bottle forceps for handling preserved specimens.
14. Killing agents for euthanizing specimens.
15. Small forceps, probes, and dissecting needles for positioning specimens in the hardening tray.
16. A variety of syringes and needles for administering killing agents and injecting fixative into specimens.
17. A spoon (teaspoon) for handling aquatic larvae.
18. Scissors—small sharp scissors for cutting specimens, and bandage scissors for cutting tags, paper, and cheesecloth.
19. Scalpel handle and a variety of blades.
20. Skinning knife (for preparing large specimens).
21. Separate set of "clean" tools (scissors, forceps, probes, scalpels, and containers) for taking tissue samples, and a supply of 95% ethyl alcohol for cleaning tools between tissue samples.
22. Thread for sewing parts back on specimens.
23. Extra tag string for restringing tags (when you discover that someone else has tied the wrong ones on the specimens).
24. A hand lens (10x) for specimen identification.
25. A color chart.
26. Scales (spring or digital).
27. Chemical splash goggles that meet ANSI Z87.1-1989 standards.
28. Cheesecloth and plastic bags for packing specimens.
29. Neoprene gloves for handling specimens in formaldehyde.
30. Variety of sewing needles for attaching tags to limbless animals.
31. Large containers with good lids to hold specimens in fluid after tagging.

FIELD NOTES

Field notes are the critical documentation that gives validity to scientific specimens.

Writing good field notes requires patience, practice, and diligence. The standard for field notes was set by Joseph Grinnell at the Museum of Vertebrate Zoology at the University of California in Berkeley. Grinnel began writing field notes in 1908. He made his last field note entry in 1939, just five days before his death. Grinnell's techniques and methods have been described and amplified by Herman (1986).

Your field notes are extremely valuable, both for your present purposes and for future reference. Take them with you as carry-on when you travel home. Deposit the originals of your notes in a reliable museum archive, preferably along with your specimens. Never rely on tape recordings or a laptop computer for recording your field notes. No recording medium is as permanent as archival quality ink and paper (Simmons 1998). Use acid-free, 100% rag paper (or spun-bonded polyethylene sheets) in a ring binder for both field journal and field catalog. Write notes with an archivally stable ink using a technical pen (I recommend Higgins T-100 Drafting Film Ink® or Rotring® ink). Do not use a disposable pen.

Ecuador: Esmeraldas Prov.

21 July 1986 (continued)

and arrived in Esmeraldas in the early afternoon. Managed to catch the bus for Borbón and get all our gear aboard. The bus is open — no windows or doors. Ana rode below, but Mark, John, and I elected to travel up top with the gear. Very windy, but great view. At dusk, the driver had us move below with a caution about insects and low branches in the dark.

We arrived in Borbón at 9:30 pm, contacted Patricio at the Ministerio de Agricultura y Ganaderia, and put up in a noisy residencia for the night.

22 July 1986

Patricio put us in contact with a reliable river man, Jorgé Villareal, who agreed to a week-long contract to take us up river, to Concepción.

Purchased supplies in the morning, then loaded the canoe and motored up the Río Santiago to Concepción. It was a beautiful, sunny day for travel. As the dry season gets underway, the water level is already dropping. We counted fish weirs along the bank, and large logs floating past us downstream to the sawmills.

Arrived at Concepción (at the confluence of Río Santiago, Río Bogotá, and Río Huimbicito) late in the afternoon. Jorge helped us make a deal to rent the upstairs of a house, and arrange for a local woman to cook for us. Ana, Mark, John, and I made the rounds of local homes and shops, asking about turtles in general and <u>Rhinoclemmys</u> in particular. We walked trails near our rental house after dark, finding only a few <u>Eleutherodactylus</u>. The full moon was probably too bright for frogs. We could hear a faint frog chorus

Figure 3. Field Journal

PPR

14 March 1988

Peru: Loreto: Selva Verde, jct. Río Chico and Río Amazonas
2° 55' 36" S; 75° 36' 24" W, 180 m

2841- Hyla
2847 PPR. Calling from low vegetation (0.25 - 0.5 m above water) at temporary pool. By night, dorsum pale yellow; by day dorsum tan to brown with darker brown markings. Flanks dull cream. Dorsal surface of thighs tan; anterior and posterior thighs black with orange and yellow spots. Suborbital and rostral stripe golden cream; throat yellow; belly dull cream; webbing grey with silver flecks. Iris reddish bronze. PPR 2841 CT 336/12-15.

2848- Phrynohyas venulosa
2850 Julio Sosa. Calling from vertical branches of tree 1.0 to 3.0 m above water by night over temporary pond. Dorsum uniform tan, brown, and with pale brown markings. Venter creamy yellow. Iris gold with black radiations. Several males were observed engaging in combat for perch sites. PPR 2848 CT 336/16-17; PPR 2850 Tape 58/4.

2851- Hydrolaetare schmidti
2852 PPR and Idoia Hidalgo. Sitting in shallow water at the edge of the river at night. Dorsum dull olive with median dorsal brown blotch bordered by reddish tan. Labial and canthal red; limb spots and flank spots dull red. Posterior thighs with yellow spots. Chin white, remainder of venter creamy yellow with dark brown reticulations. Iris silvery gray with fine black lines and horizontal brown bar. PPR 2851 CT 336/18-21. Tissues (liver and thigh muscle).

2853 Boa constrictor
Purchased in market. Seller reported that the snake was found

Figure 4. Field Catalog

Field notes should be clear and legible—printing is preferred over cursive writing.

Field notes consist of two separate but related documents—the field journal and the field catalog.

Field Journal
The field journal is a narrative account including the detailed trip itinerary, weather, rainfall, temperature, sunlight, moonlight, observations on habitat, behavior, and descriptions of collecting and preparation techniques. It is important to use a format that organizes the information in a clear, logical arrangement. Herman (1986) recommends a good standard format; the format I prefer is shown in Figure 3. A preface to each field trip should explain which chemicals were used to euthanize and preserve specimens. Any deviation from this standard technique should be recorded in the entry for the particular specimen(s). Each page of the field journal should be headed with the date and locality.

Field Catalog
The field catalog is a list of specimens collected and their field numbers, with detailed descriptions and collecting information. Herman (1986) recommends a good standard format; the format I prefer is shown in Figure 4. Each page of the field catalog should be headed with the date and the complete collecting locality, including GPS data if available. For each entry, record the field number and scientific name of each specimen. Use a single, sequential set of field numbers throughout your career—never reuse field numbers. List collectors by name or initials. If using initials, include a list of collector names. Next, describe where the animal was found (microhabitat) and what it was doing. Describe the specimen carefully, paying particular attention to details that will be lost with preservation, such as live weight and colors. Use a standard reference color chart. Hess (1988) and Zucker (1988) both recommend the Munsell System; Tucker et al. (1991) provides a comparison of several color charts. Use good quality spring scales (e.g., Pesola®) or portable electronic scales for determining weight (calibrate the scales before taking them into the field). Include references to any photographs, recordings, tissue samples or other related data.

PROCEDURE FOR PROCESSING SPECIMENS IN THE FIELD

The recommended basic field procedure is the following:

1. In a comfortable work area, assemble the field kit, field catalog, photographic equipment, tissue sampling equipment, and specimens to be processed. Make sure you have any data from other collectors necessary for the complete catalog entry for each specimen.
2. Arrange the specimens in the proper order for cataloging. This order may be based on collecting site, species, time of collecting, collector, or several of these factors.
3. Take pictures of those specimens you wish to photograph, before they are euthanized (see *Photography*).

4. Select those specimens you wish to take blood or tissue samples from before you began euthanizing specimens, as these will require special handling (see *Blood and Tissue Samples*).
5. Kill and process the specimens individually, making a careful entry for each individual in the field catalog as you go (see *Field Catalog*).
6. Lay out the specimens in the hardening trays in the order in which they are cataloged (to facilitate tagging later). If necessary, the specimen may be tagged prior to being positioned in the hardening tray, although this is not recommended.
7. Always clean up any chemical spills promptly. Clean and put away all preserving equipment and rinse out collecting bags at the end of each preservation session.
8. Write your field journal (see *Field Journal*) immediately after the specimens are preserved.

Killing

Specimens should be dispatched quickly by a method that will cause minimal pain and leave the specimen relaxed (Anon. 1987). Most field workers based in the United States will be required to use procedures in compliance with the standards of the *Institutional Animal Care and Use Committee Guidebook* (see *Appendix III)*.

Reptiles

The preferred method of killing reptiles is by injection of aqueous sodium pentobarbital (e.g., Nembutal®, Fatal Plus®) into the heart (Karlstrom and Cook 1955, Lowe 1956). Use 1.0 to 5.0 cc for large specimens, less than 1.0 cc for smaller specimens (Lowe 1956). The solution may be diluted 1:5 to 1:10, depending on the size of the specimen. The heart may be located in most reptiles by gentle pressure from one or two fingers (not your thumb) on the chest. As a general rule, in lizards the heart is just below the junction of the arms in the middle of the chest. In most snakes, the heart is about 1/4 of the way down the body from the head. Another method is injection of sodium pentobarbital or lidocaine into the brain via the upper inside surface of one of the nares (L. Vitt, pers. comm.).

Other Methods for Killing Reptiles

- Injection of MS 222™ (also called tricaine, tricaine methanesulfonate, or Finequel™) (Karlstrom and Cook 1955, O'Shea 1992). Use about ½ teaspoon in 20 ml of water to make an injectable solution (L. Grismer, pers. comm.). Use a freshly mixed solution—it looses effectiveness after a few days.
- Injection of Lidocaine™, also known as lidocaine hydrochloride, 2-diethylamino-N(2,6-dimethylphenyl) acetamide, Anestacon™, Dilocaine™, L-Caine™, Lida-Mantle™, Nervocaine™, Octocaine™, and Xylocaine™.
- Livezey (1958) recommended the use of procaine hydrochloride for reptiles and amphibians. Other names for this drug include procainamide hydrochloride, Procan™, Procanbid™, and Pronestyl™. A 10% mixture is prepared

by dissolving tablets (which contain 0.07 g of procaine hydrochloride) or crystals in water, or by obtaining the drug as a prepared solution. About 0.05 cc per gram of body weight should be injected into the pleuroperitoneal cavity just posterior to the heart.

- Lambert (1967) suggested using Anectine™ (succinylcholine chloride). Other names for this drug include diacetylcholine chloride, Quelicin™, and Sucostrin™. It is available as a white powder. The recommended solution is 25 mg/cc of water. Inject into the abdominal and pericardial cavities. Small reptiles require 0.05 to 0.20 cc, large reptiles require up to 2.5 cc or more.
- Reptiles may be euthanized by placing them in a killing jar containing cotton soaked in nail polish remover (ethyl acetate; see O'Shea 1992), or acetone (L. Grismer, pers. comm.).
- Application of a small amount of benzocaine (ethyl p-aminobenzoate) based oral analgesic (e.g., Oragel®) to the head (Altig 1980) or venter (Chen and Combs 1999) may be used to kill small, thin-skinned reptiles.
- Chloroform.
- Ether.
- Less desirable methods of killing include the injection of isopropyl alcohol into the brain through the optic foramen, or by freezing (Meylan 1989). The drawbacks to these two methods are that you may damage the cranium of the specimen by the former, and the latter may result in a frozen specimen. Specimens that have been frozen do not preserve well, due to ruptured cells and the inability of fixatives to penetrate frozen tissues (see *Frozen Specimens*).

Amphibians

The preferred method for killing amphibians is to relax them in a solution of hydrous chlorobutanol (1,1,1-trichloro-2-methyl-2-propanol; also known as Chloretone™; see Snyder 1915). Prepare the killing solution by making a saturated solution of hydrous chlorobutanol crystals dissolved in 95% ethyl alcohol; use a few milliliters of this saturated solution in about 500 ml of water. Alternately, a killing solution may be prepared by dissolving one teaspoon of hydrous chlorbutanol crystals in one liter of water (McDiarmid 1994).

Other Methods for Killing Amphibians

- Application of a small amount of benzocaine (ethyl p-aminobenzoate) based oral analgesic (e.g., Oragel®) to the head (Altig 1980) or venter (Chen and Combs 1999).
- Submersion in a 10% ethyl alcohol and water solution.
- Submersion in solution of procaine hydrochloride. Refer to the recommendations of Livezey (1958) described above.
- Use of a killing jar containing cotton soaked in nail polish remover (ethyl acetate; see O'Shea 1992).
- Very large amphibians, particularly those with thick skins, may be killed with an injection of sodium pentobarbital or any other of the methods described above for reptiles.

Blood and Tissue Samples

Although some success has been achieved in extracting DNA from formaldehyde fixed specimens preserved in ethanol (Chatigny 2000), it is preferable to prepare fresh blood or tissue samples expressly for this purpose. Non-fatal techniques for collecting blood or tissue samples from live amphibians and reptiles are described in the following:

Tissue. Carapace notching (Mockford et al. 1999), caudal muscle plug (Haskell and Pokras 1994), shed skin (Bricker et al. 1996, Clark 1998), autonomized tails (Cameron et al. 1998, Cordero et al. 1998), amphibian toe clips (Gonser and Collura 1996).

Blood. Techniques for lizards (Esra 1975), cervical sinus of turtles (Bennett 1986), coccygeal vein (Haskell and Pokras 1994), caudal puncture in turtles (Powell and Knesel 1992, Stephens and Creekmore 1983), neck puncture in turtles (Wibbels et al. 1998), cardiac puncture in turtles (Maxwell 1979), cardiac puncture in snakes (Branch 1973, Sooter 1955), caudal puncture in snakes (Reinert and Bushar 1991).

Tissue Samples from Euthanized Animals. The following instructions for taking and processing tissue samples are based on the recommendations of Dessauer et al. (1996).

First, make sure that your permits allow you to take tissue samples. Although most jurisdictions consider tissues to be the same as specimens, in some areas, special permission is necessary to take tissues.

Take tissue samples immediately after death. Any delay in sampling will result in degraded DNA. Maximize the diversity and quantity of tissue types sampled (e.g., blood, heart, intestine, kidney, liver, skeletal muscle, stomach, and snake venom). Each sample should be at least the size of a pencil eraser. Immediately after tissues are extracted and placed in containers, protect them from light. Put the tissues in a cold and dark environment as soon as possible. Note in the field catalog which tissues were removed from each specimen.

The tools, containers, and reagents used to collect samples for DNA analysis must be kept very clean. Rinse the forceps, scissors, and scalpel blades immediately in clean water (before the blood on them dries), then clean them in 95% ethyl alcohol before taking each sample.

Using clean tools, cut through the skin of the animal's left thigh and remove a chunk of muscle at least the size of a pencil eraser. Do not cut the femoral artery. If the animal is legless, take the sample from the muscle from along the vertebral column or the neck.

Make a small lateral incision in the left side of the abdominal cavity. With small forceps, grasp the liver and pull it through the opening. Remove a piece about the size of a pencil eraser, and place it in an appropriate, labeled container. Use a similar procedure to sample other organs as desired.

Place each tissue sample in an appropriate, labeled container, and clean the tools. The preferred way to preserve DNA samples is to immediately freeze them in liquid nitrogen. This requires hauling around an appropriate container and finding a source of liquid nitrogen, which may be difficult to do in the field. Tissues in liquid nitrogen should be placed in (in order of preference) (1) plastic cryotubes or (2) or wrapped tightly in extra heavy-duty aluminum foil. Tissues may also be put in polyethylene bags (with air removed), but plastic bags should not be submerged in liquid nitrogen.

Label each tissue sample immediately with the specimen's field number written in pencil on a small piece of acid-free 100% rag paper or spunbonded polyethylene material placed in the tube, vial or bag with the tissue sample. If using plastic cryotubes, scratch the field number on the tube with a needle, and label the tube with a cryomarker.

If it is not possible to freeze tissues, they may be preserved in (1) 95% ethyl alcohol for 1-2 hours and then placed in fresh 95% ethyl alcohol; (2) 75% ethyl alcohol for 1-2 hours and then placed in fresh 75% ethyl alcohol; or (3) isopropyl alcohol instead of ethyl alcohol. A solution of 95% ethyl alcohol is vastly superior to 75%. Use at least twice the volume of alcohol as the volume of tissue sample. Cut the tissue sample into pieces 2-4 mm in diameter. You may add 100 μmol of EDTA (ethylenediamine tetra-acetic acid) per liter of alcohol for stabilization. Tissues in alcohol should be placed in glass vials with good closures. Wrap the closure/container junction with masking tape. Protect tissue samples from light, and move them to a dark, cool environment as soon as possible.

The use of buffers such as DMSO (dimethyl sufoxide) is not recommended for tissue collection or storage. They do not provide long-term stability for storage of tissues.

After being frozen in liquid nitrogen in the field, tissues may be transported back to the museum in liquid nitrogen or on dry ice (solid carbon dioxide). It is legal to ship a properly labeled, non-pressurized metal container of liquid nitrogen by air, but it will be far easier to use a "dry shipper," which contains an absorbent material that keeps the liquid nitrogen from spilling.

In the laboratory, store tissue samples in an ultracold freezer (-70°C to -150°C), on dry ice, or in liquid nitrogen.

Fixation and Preservation

Most specimens of amphibians and reptiles are preserved in fluid. Historically, specimens were cut open and placed in containers of 70% ethyl alcohol for preservation (Simmons 1991, 1993). The current two-step process of formaldehyde fixation followed by alcohol preservation began in the late 1890's or early 1900's, after the discovery of the fixative properties of aqueous formaldehyde (Ford and Simmons 1997). Fixation followed by preservation did not become common as a field technique until after the turn of the century. There is a widespread belief among herpetologists that formaldehyde fixation is necessary for the successful preservation of specimens, but the oldest fluid preserved herpetological specimens in museums were not fixed in formaldehyde. In the long term, we know that alcohol causes tissues to shrink and colors to fade, due to the loss of pigments and to structural changes caused by shrinkage or swelling in preservatives (Simmons 1995). We also know that formaldehyde fixation disrupts protein chains, making it difficult to extract DNA from the tissues, causes specimens to darken, and over time, will decalcify bone (Simmons 1995, Stuart 1995). We know very little about the long-term stability of formaldehyde fixed specimens because no studies have been conducted (Simmons 1991, 1993, 1995).

Fixation is a chemical process that (1) prevents autolysis (the degradation of proteins into amino acids) by the formation of covalent bonds known as crosslinks, and (2) coagulates cell contents into insoluble substances (Simmons 1995, Stoddard 1989). A preservative does not form crosslinks but disorders proteins by altering patterns of hydrogen bonding. Preservatives have been in use for fluid specimens for about 350 years, fixatives for about 100 years.

To be successful, a fixative must penetrate the tissue rapidly and be slightly acidic. The most widely used fixative for herpetological specimens is formaldehyde. Formaldehyde is sold as an aqueous solution of 37% v/v (or 40% w/v) formaldehyde with a small amount of methanol (6-15%) added to the solution to prevent polymerization. The term *formalin* refers to this aqueous mixture of 37% commercial formaldehyde. The traditional "10% formalin" used to preserve reptiles and amphibians is made by diluting one part commercial formaldehyde with nine parts water, thus "10% formalin" is actually 3.7% formaldehyde in aqueous solution.

When diluted with water, formaldehyde solutions are acidic, in the range of pH 3.0 to 4.6. This is because of the presence of small amounts of formic acid, either as an impurity, remaining from manufacture, or as a result of the oxidation of part of the formaldehyde. Formaldehyde reacts with oxygen in the air to oxidize into formic acid, so it has to be neutralized or buffered. The use of unbuffered formaldehyde will shorten the useful life of the specimen and may cause problems with later attempts to prepare the specimen for other types of study (Quay 1974). The pH range achieved by buffering is critical—decalcification starts at pH 6.4 and below; but the clearing of tissue starts at pH 7.0 and above.

The traditional buffers for formaldehyde are borax and calcium carbonate. Neither of these should be used as they are not stable for very long (Hughes and Cosgrove 1990, Simmons 1995). The preferred buffer for formaldehyde is that proposed by Quay (1974): use 4 g of monobasic sodium phosphate monohydrate ($NaH_2PO_4H_2O$) with 6.5 g of dibasic sodium phosphate anhydrate (Na_2HPO_4) per liter of solution consisting of one part commercial formaldehyde (37%) and nine parts of distilled or deionized water. These two salts can be pre-measured and sealed in small plastic packets, ready for use in the field. When mixing fixative solutions, always measure the water and formaldehyde with a small plastic graduated cylinder. Do not try to mix solutions by attempting to read a meniscus on a quart or liter size container.

Because formaldehyde is prone to oxidation, it should be mixed with distilled or deionized water. Tap water usually contains unwanted chemicals that will affect the quality of the mixed solution. However, in the field it is often necessary to use local water sources.

Formaldehyde Safety

Safety precautions must be observed when working with formaldehyde both in the field and the laboratory. Most people who work with formaldehyde for any length of time develop sensitivity to it. Formaldehyde is an irritant to the skin and the respiratory system and presents a cancer risk with improper exposure (Council on Scientific Affairs 1989, Gibson 1983, OSHA 1992, Turoski 1985). According to established safety standards, if you can smell formaldehyde, you are receiving an unsafe dose (OSHA 1992).

- Use formaldehyde only in a well-ventilated area.
- Always wear eye protection (splash goggles that meet ANSI Z87.1-1989 standards) while working with formaldehyde. It is easily absorbed by mucous membranes, and can cause serious damage if splashed in the eye.
- Do not wear soft contact lenses when working with formaldehyde. Soft lenses can absorb formaldehyde and trap it against the eye (Cohen et al. 1979).
- Wear gloves made from Neoprene™ or other material rated for formaldehyde resistance. (Refer to *Appendix III* for sources of safety equipment, including rated resistant gloves and eye protectors). Do not use latex gloves—formaldehyde readily penetrates latex (Raloff 1985).

Bear in mind that we are already exposed daily to formaldehyde in a wide variety of common products ranging from cosmetics to cigarettes to plastics.

Paraformaldehyde

The solid polymer of formaldehyde, called paraformaldehyde, may be carried in the field as a dry powder, flakes, or pellets. Taub (1962) suggested mixing paraformaldehyde with sodium hydroxide in the field, but hot to boiling water is needed to dissolve the paraformaldehyde, and results in a formaldehyde solution with a pH of 12. Huheey (1963) suggested replacing the sodium hydroxide with sodium carbonate, which eliminates the need to boil the solution, but produces formaldehyde with a pH of 11.6, which is still so alkaline that it will cause specimens to clear. Nelson and Sparks (1999) cautioned that if Alconox® is used to dissolve paraformaldehyde, the solution may cause specimens to clear, loose pigment, and swell. These problems might be avoided by a thorough rinsing between fixation and preservation in alcohol.

Ehmann (1989) suggested preparing two dry solutions in the lab in plastic bags ready for mixing in the field. In one bag place 80g of paraformaldehyde flakes or powder, 20 g of anhydrous sodium carbonate, and 0.5 g of a wetting agent in solid form (e.g., a detergent); in the second bag put 21 g of citric acid. In the field, mix the contents of the first bag with 2 liters of water, stirring constantly to prevent clumping. Agitate the solution occasionally over the next four hours at 20-30°C to dissolve the contents. Once the paraformaldehyde is dissolved, add the citric acid and stir until no carbon dioxide evolves (about five minutes). Allow gas to continue to escape from the solution for 24 hours. This will result in a "neutralized" solution of 10% formalin; however, depending on the wetting agent used, it may or may not contain chemical contaminants that could cause problems in later use of the specimens.

Preservation

Good preservation depends on the rapid penetration of the fixative (usually formaldehyde) or the preservative (usually ethyl alcohol) into the tissues. Four basic methods are used to introduce the fixative or preservative solution into the animal.

1. ***Opening the Body Cavity***: The oldest method is to make cuts into the body cavity and into large muscle masses, particularly on the limbs, to allow the chemicals to penetrate. This method is not desirable because of the damage it does to the specimen, but may be used in an emergency when no other option is possible.
2. ***Perfusion***: Particularly if formaldehyde fixation is not utilized, specimens should be perfused (Jones and Owen 1987, Quay 1974, Schultz 1924). Perfusion requires that two cuts be made into the body. One cut is made for access to the left femoral artery or the left ventricle of the heart for injection of fixative or preservative. A second cut is made to open a vein to provide an exit for the fluid. Insert a large (18-24 gauge) needle into the artery, hold it firmly in place, and apply gentle pressure. Force fluid through the system until the effluent is clear, or without blood. A large syringe (20-50 cc capacity) holds sufficient fluid to perfuse most small amphibians or reptiles

without refilling. For larger animals, a valve may be used to permit refilling of the syringe without allowing air to enter the system, or a pump may be used to draw solution from a large container. Perfusion is the best method to distribute a chemical quickly and evenly through a specimen. The drawbacks include that two cuts must be made in the animal; the blood is removed; and the process takes a little longer than standard injection (see *Injection*).

3. ***Permeation***: Smaller specimens may be placed in the proper position directly on formaldehyde-soaked paper towels for hardening, then covered with more paper towels and a volume of formaldehyde (see *Hardening and Tagging*). Sufficient formaldehyde will penetrate the skin of the specimen rapidly. Larger and thicker-skinned specimens must be injected with the fixative (see *Injection*). As a general rule, any amphibian smaller than five inches long (snout-vent length) should not have to be injected or perfused for the formaldehyde to penetrate the body tissues, except for gravid females. Most reptiles must be injected. Of the fixatives currently available, only formaldehyde has sufficient power of penetration to be used with whole animals. Standard histological fixatives, such as gluteraldehyde, are inadequate for permeation of whole animal bodies (Dempster 1960, Hopwood 1973, Stoddard 1989).
4. ***Injection***: Injecting the fixative solution into the body cavity and the major muscle masses is the standard procedure now in use for nearly all reptiles and for larger amphibians, although perfusion provides better circulation of the fixative or preservative solution. Do not inject too much formaldehyde in any one spot. Its ability to penetrate tissue is quite good, once it is through the skin. Syringe needles may be modified for more rapid injection of fixative by attaching a feed tube, using a pump action spray bottle or hand pump (Congdon et al. 1975, Jackson 1971, Johnson and Parker 1980, Saul 1982), a small electric pump, or a garden sprayer (Forstner et al. 1997). Inject as follows:

   Reptiles. One of the 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 (L. Vitt, pers. comm.). Geckos and other specimens that are coated with a thin, waxy film may pose problems for the penetration of fixatives, and the separation of the stratum corneum from the lower epidermal layers. Such specimens may first be soaked for a couple of minutes in a 70% ethyl alcohol solution to remove the waxy outer coating, then placed in the hardening tray (L. Grismer, pers. comm.), or a small series of perforations with a sharp needle may be made on the limbs, body and tail (L. Vitt, pers. comm.).

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 base to 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. Use fairly shallow trays with well-fitting lids. I prefer trays made of polypropylene with snap-top lids. These are relatively unbreakable, resistant to fixatives and preservatives, and keep the formaldehyde fumes contained. Do not use metal trays, because the formaldehyde will oxidize the metal and cause the specimens to discolor. 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 with only water for this step. Although this is a way to avoid contact with formaldehyde vapors, there are two serious drawbacks. When covered with paper towels, the specimens may jerk and twist into contorted positions as the formalin solution is added. The second drawback is that the paper towels moistened with water will dilute the formalin.

In an emergency, where no hardening tray is available, specimens can be fixed and hardened in plastic bags (Hahn 1959). A hardening tray for very large specimens can be fashioned from a large sheet of plastic, using boards or bricks to hold the sides, by lining a pit in the ground with a plastic sheet (Darovec 1979), or by lining a cooler with plastic (L. Grismer, pers. comm.)

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 a container without tangling up with other specimens; and (4) packed for shipping without damage to digits or tails (Figure 5). 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.

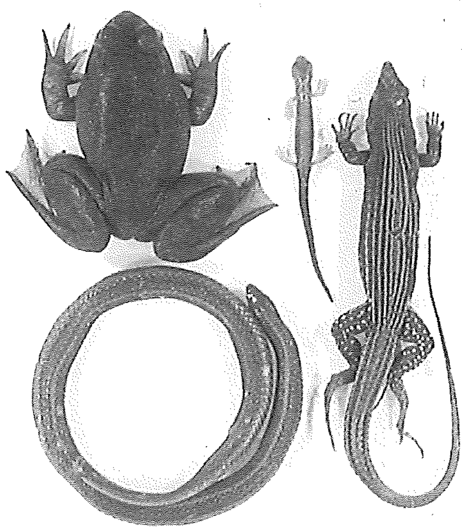


Figure 5. Positioning specimens for hardening.

Metamorphosed amphibians are positioned in the hardening tray on their venter, with limbs positioned in a natural, relaxed manner (see Figure 5). Large limbless amphibians (e.g., caecilians) may be positioned as are snakes (see *Snakes*); long amphibians (such as certain salamanders) may be positioned as are lizards (see *Lizards*).

Amphibian larvae and eggs should be placed in a vial or flat-bottomed jar with the buffered 10% formalin solution and the field tag (Bragg 1949). Lay the container on its side if necessary so that the specimen will harden in a flat position. Do not overcrowd specimens. When eggs are attached to vegetation, include a small piece of the vegetation with the eggs as well. Removing the adherent vegetation will damage the eggs, and it is important to know whether the clutch is affixed to a stem or leaf.

Lizards are placed on their venter with limbs flexed in a natural position (see Figure 5). The dewlap, if present, may be extended to one side and held in place with the lizard's hand while hardening. The caudal appendage of long-tailed individuals may be curled up around the body.

Snakes are coiled clockwise with the head to the outside. Snakes too large to coil in the bottom of a standard size jar should be coiled up the inside of a container the same size as a standard storage jar, with the head on top.

Turtles should have the limbs, head and neck, and tail extended from the shell. The mouth should be held open during hardening with a piece of soft wood, plastic, or cork. It is sometimes necessary to pin the limbs and neck in position until hardened.

Very large reptile specimens are usually too expensive to preserve whole. If this is the case, the body should be carefully measured, and the skin removed, preferably with the head and tail entire. The remaining body should be roughed out (gutted and major muscle masses removed) and dried for later preparation as a skeleton. Take careful notes on stomach contents, guts, reproductive state, and size of fat bodies before disposing of the remains. For hardening, lay the skin out flat, dorsal side down. Cover the entire inside surface (including inside the limbs) with a layer of cheesecloth, which will serve as a wick to draw the fixative or preservative in. Roll the skin up loosely and submerge it in the fixative or preservative solution. An alternative is to disarticulate the specimen and preserve it in pieces (Cook 1965).

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, caecilians, 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.

For eggs or larvae, place a tag in a vial with the specimens so that the field number may be read from the outside. Tape the vial/lid junction with masking tape to keep the lid from becoming loose during transport.

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 packing or further processing. Leaving 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 (Stuart 1995).

PACKING AND TRANSPORT OF SPECIMENS

> Sad experience taught us but too late, that from the sultry humidity of the climate, and the frequent falls of the beasts of burden, we could preserve neither the skins of animals hastily prepared, nor the fishes and reptiles placed in phials filled with alcohol.
>
> —Alexander von Humboldt, 1889

After specimens have been in formaldehyde for a week to ten days, they may be packed for shipment. Lay specimens out on a length of cheesecloth as described for packing specimens for loans (see *Procedures for the Preparation of Outgoing Loans*). Keep the size of the package per plastic bag small. Packing will be easier, and loss to the collection should leakage occur will be reduced. Do not leave standing liquid in the plastic bags. Double bag each package, closing the tops of the bags securely. Include an address label between the outer bag and inner bag of each package. Label each bag as "Preserved scientific specimens—No commercial value."

Larvae, particularly small larvae, should be transported in liquid. Transfer the specimens to the smallest size vial that will hold them without causing deformation. Diluted formalin or water may be used if shipping by air (never put larvae in alcohol solutions). Fill the vial completely with the liquid and secure the lid tightly to prevent leakage. Pad the vials with paper towels, and enclose them in a plastic bag. Label each bag as "Preserved scientific specimens—No commercial value." Immediately upon arrival, transfer the larvae to larger vials and a fresh buffered 10% formalin solution.

Ship specimens by air whenever possible to save time. Upon arrival, specimens should be unpacked and prepared for permanent storage (see *Part IV, Museum Collections*).

PREPARING SPECIMENS FOR SKELETONIZING

Reptile specimens to be prepared as skeletons by the use of arthropods (e.g., dermestid beetles) should be roughed out (gutted and most muscle mass removed) and the carcasses dried rather than formaldehyde fixed. One way to easily desiccate a carcass is to hang it in a wire basket in the engine compartment of a field vehicle (Bond 1939) or dehydrate it in ethyl or isopropyl alcohol (L. Grismer, pers. comm.). Amphibian specimens should be preserved but not desiccated before skeletal preparation (Simmons 1986a). Skeletons should be prepared of properly identified individuals with good locality data. Record the animal's sex, snout-vent length, breeding condition, and any other pertinent information in the catalog.

EMERGENCY SUBSTITUTES FOR FIXATIVES AND PRESERVATIVES

Sometimes you have the opportunity to preserve some particularly desirable specimens, but you can't get formaldehyde. In this case, 70% ethyl alcohol is the most desirable substitute, and second choice is 40-50% isopropyl alcohol. Methyl alcohol does not make a satisfactory preservative (Simmons 1995). If neither ethanol nor isopropanol is available, try a strong, inexpensive liquor. I have been given collections in Ecuador and Paraguay that were preserved with the local aguardiente (corrupted from sugarcane, flavored with anise, alcohol content around 40%). In both instances, the specimens had a rather distinctive odor, but they certainly qualified as preserved. In general, an alcohol solution that will burn when contacted by a flame should be strong enough to preserve a specimen, if sufficient penetration of the preservative can be achieved.

COLOR PRESERVATION

Both reptiles and amphibians will fade after either fixation in formaldehyde or preservation in alcohol. Several formulas to better retain colors have been proposed, but none has been proven effective in the long term, either for color preservation or specimen preservation. For recipes see Guerra (1976), Waller and Eshmeyer (1965), White and Peters (1969), and Windsor (1971). Colors can also be preserved by preserving the skin as a dry preparation (see Beebe 1947, Juszcyk 1952, Kincaid 1948, Paranjape and Mulhekar 1979, Schueler 1981, and Taylor 1981b).

FROZEN SPECIMENS

Once a specimen has been frozen, it is not possible to make a satisfactory fluid preserved preparation from it (Scott and Aquino-Shuster 1989). Freezing causes cells to rupture, weakening the tissue (Mills 1975). Neither fixatives nor preservatives can penetrate tissues while they are frozen. Frozen specimens should be prepared as skeletons. If it is absolutely necessary to make a fluid preparation from a frozen specimen, thaw it in a solution of buffered 10% formalin, injecting it as thawing permits.

MISCELLANEOUS PRESERVATION TECHNIQUES

General preservation techniques for amphibians and reptiles. Casas-Andreu et al. (1991), Scrocchi and Kretzschmar (1996).
Specific Types of Preparation. Embedding in plastic (Robbins 1976), embalming (Elkan 1970), freeze drying larvae (Altig (1975), hemipene preparation (Dowling and Savage 1960, Klauber 1972, Ortenburger 1923), paraffin infiltration (McCoy 1929, Noble and Jaeckle 1926).

Skeletal Preparation. Skinning a giant tortoise (Anon. 1899); use of dermestids (Jannett and Davies 1993, Sommer and Anderson 1974, Tiemeier 1940, Valcarcel and Johnson 1981, Vorhies 1948); use of dermestids for specimens previously preserved in fluid (De la Torre 1951, Dirrigl et al. 1993, Gritis and Brunner 1990); use of clothes moth larvae (Banta 1961); mealworms (Allen and Neill 1950), terrestrial isopods (Maiorana and Van Valen 1985). Maceration (Hill 1975), preparation of turtle shells (MacMahon 1961), amphibians (Simmons 1986a—do not use bleach as recommended in this paper).

Clearing and Staining. Dingerkus and Uhler (1977), Potthoff (1984), Springer and Johnson (2000), Taylor (1967a, 1967b); Taylor and Van Dycke (1985), Wassersug (1976).

Part IV. MUSEUM COLLECTIONS

> A major systematic collection of amphibians and reptiles is like a three-way hybrid between a pickle warehouse, a reference library, and a mail-order establishment.
> —C. J. McCoy (1981)

The most important functions of collection management are (1) maintaining the integrity of the specimens and their associated data; (2) maintaining the specimens and data in optimum usable condition; and (3) making the specimens and data available for appropriate use.

Collections are useless if the specimens sit out the passing decades untouched on dusty shelves. To be valuable resources, specimens must be made available to the scientific community, while at the same time the integrity of the collection is maintained. Data files should be cross-referenced and kept up to date with the addition of specimens and refinement of specimen data. The storage environment and the physical condition of the individual specimens must be monitored regularly.

In order to maintain the integrity of the specimens and data, standardized procedures should be established, documented, and implemented for the various tasks undertaken in the course of management. The use of standardized procedures will insure that different individuals working with a collection will be able to maintain its continuity. Write down the standardize procedures you put into place in the collection, and keep a written record of any changes in these procedures. How you do things today may well be important to someone using the collection in 50 or 100 or 200 years. Because you can never predict with certainty when you will be able to resume an interrupted procedure, it is important that it be obvious to another worker what you were doing.

CONSERVATION OF HERPETOLOGICAL COLLECTIONS

Conservation of specimens and data-bearing media has received very little attention in natural history collections until recently. Fortunately, this is rapidly changing, with new information about materials and techniques (e.g., Rose and Hawks 1995; Williams 1999) and new standards for collection care (Society for the Preservation of Natural History Collections 1994).

Conservation means preservation of a specimen in a way that retains as completely as possible its original composition while prolonging its useful life as a specimen. Conservation concerns for herpetological collections begin with the chemicals and techniques used to capture, euthanize, fix, and preserve the specimen, and continue with the collection storage environment and how the specimens are used.

Natural history specimen preservation and collection management is plagued by a long tradition of untested techniques, handed down from one worker to another, and numerous anecdotal observations that seem to explain phenomena but are, in fact, baseless. It is important to distinguish between those processes and techniques that have been appropriately tested for the long-term stability of the specimens and those for which we have only anecdotal observations. Many techniques reported in the literature appear to work because the damage they do takes 10, 20, or 30 years to become apparent. For example, consider phenoxetol, enzyme detergents, and formaldehyde. Phenoxetol was used with apparent success for ten years before its failure as a preservative was determined (Crimmen 1989). It took 20 years before the use of enzyme detergents to prepare skeletons was shown to be a destructive practice (Shelton and Buckley 1990). The use of formaldehyde fixation has never been tested against the use of alcohol preservation without formaldehyde (Simmons 1995). Many traditional collection care practices are not good for the specimens.

Fluid Preserved Specimens

We do know that the long-term stability of a fluid preserved specimen depends chiefly on three factors: (1) the quality of fixation or initial preservation; (2) post-preservation processing; and (3) the quality and stability of the storage environment (Table 3).

The biggest threats to fluid preserved herpetological specimens are inadequate preservation, loss of fluid preservative, heat, light, and ultraviolet radiation (Figure 6). Unfortunately, the choice of fixatives and preservatives relies on tradition, not empirical knowledge (Simmons 1993, 1995). Most herpetologists believe that formaldehyde fixation is necessary for the long-term preservation of specimens, but formaldehyde has only been in use since the 1890s, while the oldest known fluid preserved specimens are several hundred years old. While formaldehyde fixation is necessary for many types of histological preparations, no published controlled experiments demonstrate that formaldehyde fixation enhances long-term preservation.

Skeletal Specimens

Most skeletal preparations are either dry or in the form of cleared and stained specimens stored in glycerin. References for techniques for preparing skeletons are listed in *Miscellaneous Preservation Techniques*.

Dry skeletons are susceptible to attack from pests. Monitor skeletons regularly, as part of a program of integrated pest management (IPM). Using IPM techniques, most pest problems can be prevented or dealt with without the use of chemicals (Jessup 1995).

Skeletons should not be boiled, bleached, subjected to ammonia treatments, or treated with consolidants or whiteners. Studies have shown that all of these

Table 3. Factors determining long-term usefulness of fluid preserved specimens.

1.Quality of fixation or initial preservation	a. Time interval between death and fixation	
	b. Fixative or preservative quality	
	c. Penetration rate of fixative or preservative	
	d. Temperature during fixation or preservation	
	e. Ratio of fixative or preservative fluid to specimen	
2.Post-fixation processing	a. Washing or rinsing of specimen	
	b. Transfer to preservative fluid	
	c. Hydration and/or dehydration of specimen	
3. Storage environment	a. Temperature (set point and fluctuations)	
	b. Light (cumulative exposure)	
	c. Ultraviolet radiation (cumulative exposure)	
	d. Relative humidity (set point and fluctuations)	
	e. Vibrations	
	f. Integrity of container seal	i. Evaporation
		ii. Contamination
	g. Handling of specimen	i. Physical damage
		ii. Dehydation
		iii. Rehydration

treatments will cause premature aging of the bones, and may interfere with chemical analysis such as DNA extraction (Caldararo and Grabow 2000, Shelton and Buckley 1990, Williams 1999). Sometimes bones become greasy or chalky in storage. Chalkiness results from prior treatment with enzymes, bleach, ammonia, or other chemicals that were not adequately rinsed out of the bone. There are no treatments to correct this deterioration. The grease that migrates to the surface of bone is a natural component of the bone. Some animals, such as sea turtles, have particularly greasy bones. Bones are almost always degreased for cosmetic purposes. Degreasing is an unnecessary treatment that damages bone. Typical degreasing treatments include boiling the bones, subjecting them to ammonia baths, and boring holes for the penetration of gasoline. All of these damage the bone and reduce its scientific usefulness.

Figure 6. Specimen damage from loss of fluid.

In some instances, grease accumulation on the surface of bones may make some features difficult to see. Acidic or abrasive airborne pollutants may stick to surface grease (causing damage to the bone), and the grease may attract pests. When grease presents a problem, clean the bone surface only, using a solution of 95% ethyl alcohol and clean polyester fiber or a clean, double-washed soft cotton cloth. Use Q-Tips® for small areas. If the grease returns another surface cleaning can be done.

The concerns for glycerin preparations are similar to those for other fluid preserved specimens, with a couple of added precautions. Glycerin is hygroscopic, so it absorbs moisture from the air. If the containers are not tightly closed, in high humidity conditions the glycerin may absorb sufficient water to overflow the container, and result in a perilously dilute solution. Glycerin absorbs atmospheric pollutants and contaminants that may cause the solution to become too acidic or alkaline to serve as a good preservative. Glycerin is an excellent growth medium for bacteria and mold. Containers of glycerin preparations must be well sealed, and a few crystals of thymol ($C_{10}H_{14}O$) should be added to each container to prevent bacteria and mold growth (see *Procedures for Handling Specimens in Glycerin*).

The Storage Environment for Herpetological Collections

The single most important factor for the longevity of natural history collections is their storage environment. This means maintaining the most stable temperature and relative humidity possible (with the smallest fluctuations from set points), and protecting the collection from light and ultraviolet radiation. In general, a temperature of 65-70°F (18-21°C) and relative humidity of 47-55% is preferred for the storage of natural history collections (Bachmann and Rushfield 1992, Appelbaum 1991).

The optimum temperature for storing fluid preserved specimens—cool enough to reduce the flash point and evaporation of the preservative without affecting preservative or specimen quality—is 65° F (18° C) at 50% relative humidity (Simmons 1995). Higher temperatures cause faster rates of the chemical processes of degradation (Rose and Hawks 1995, Thompson 1986); lower temperatures may cause problems with preservative quality (Simmons 1995).

Collections should be protected from fluctuations in temperature and relative humidity. All materials in the collection are affected by these fluctuations—the paper that the field notes, labels, and catalogs are written on expands and contracts, breaking along binding seams; skeletal material is stressed; teeth fall out of skulls; jar lids become loose; the emulsion peels loose from photographs; inks fade (Browning 1970, Shahani and Wilson 1987, Thompson 1986). Relative humidity and temperature should be monitored in the collection area throughout the year with a hygrothermograph or datalogger. Take steps to ameliorate the environmental fluctuations that occur in collection storage.

Fluorescent lighting produces significant amounts of ultraviolet (UV) radiation. UV is very damaging to specimens. Preserved specimens should be kept in the dark whenever they are not in use. Lights in the collection storage area should be turned off when not in use. All UV sources in specimen storage or use areas, including laboratories and offices, should be equipped with UV filters (see *Appendix III* for suppliers). Glass jars are not adequate UV filters—glass allows damaging UV in the 310-400 nm range to pass through (Harris 1968; Macleod 1975). Preserved specimens should never be exposed to sunlight or ambient light coming in through a window.

Specimens in fluid should be kept in glass, stainless steel, or high density polyethylene containers with good closures. Alcohol preserved specimens are hygroscopic. When specimens are removed from their containers to be examined, they should be kept submerged in a tray of the same preservative they are stored in. Do not put preserved specimens in water for examination, as water absorption causes extreme stress to the specimen and will dilute the preservative in the container when the specimen is returned. Water absorption and subsequent dehydration in the preservative will damage preserved specimens.

Skeletons, turtle shells, slide mounts, tapes, negatives, photographs, color transparencies, and compact disks should be protected from dust and environmental fluctuations in closed containers made of acid free board, polyethylene, polypropylene, or polystyrene, or in metal cabinets that have a powder coat finish. Many dust particles are acidic or abrasive (Thompson 1986). Combined with moisture from the air or with smoke (which is oily), dust can form a coating that will etch the surfaces to which it adheres.

Because heat greatly accelerates the deterioration of cellulose (Appelbaum 1991, Banks 1978, Browning 1970), the cooler the storage temperature the better for paper, above a lower limit of 60°F (15°C). Paper-based collection records should be housed in a stable storage environment and protected from light (particularly ultraviolet radiation) and pollutants. The ideal storage environment for most paper is 68°F (20°C) ± 4°F (2°C) at a relative humidity of 45% ± 2% (Shelley 1992, Vogt-O'Conner and van der Reyden 1996). Refer to Vogt-O'Conner and van der Reyden (1996) for a good summary of information on the storage of paper-based materials.

Important papers that are deteriorating (e.g., documents on acidic paper, mimeographed documents, self-carbonized forms) can be copied using preservation photocopy techniques. Refer to Jordan (1993) for recommendations on equipment, materials, and procedures for preservation photocopying.

Field notes and other permanent collection documentation should be protected in acid-free slipcases or envelopes (Thompson 1986). Refer to Vogt-O'Conner and van der Reyden (1996) for a detailed discussion of the storage of paper-based materials. Binding field notes with acid-free materials is acceptable only if existing holes in the pages are used. Binding methods such as the standard "library binding" (side-seam sewing) are not acceptable (Banks 1978). The paper develops a stress line where it is sewn, and with use, will break along this line. When bound field notes are opened to be read, the pages are pulled taut against the threads, cutting and tearing the paper. When the bound notes are forced down on a flat photocopier glass, the damage is even worse. Library bindings are designed to protect materials from a lot of handling over a short period of time, not for long-term archival protection of permanent documents.

Photographs, negatives, and color transparencies should be stored at 68°F (20°C) ± 4°F (2°C) at a relative humidity of 20-45% for prints and 20-30% for negatives, with fluctuations of no more than ± 3% (Henricks 1992a, Vogt-O'Conner 1997). Wear cotton gloves when handling photographs and negatives. Albright (1993) provides recommendations for proper enclosures for prints and negatives. Color slides deteriorate much faster than prints or negatives. The only way to prolong the useful life of a color slide for more than 10-20 years is to make use copies and keep the originals in cold storage. Refer to Vogt-O'Conner (1997) and Wilhelm and Browner (1993) for more information on caring for photographic materials. Store photographic materials away from light.

Recorded tapes must be stored so that the tape is not distorted. Avoid winding the tape too tightly by storing the reel after it comes off the machine at playback speed (Hendricks 1992b, Wickstrom 1982). Store tapes and other magnetic media in an area protected from particulates and extremes of temperature or rapid changes in relative humidity. The ideal storage environment for all magnetic media (e.g., tapes and computer disks) is 49-59°F (9-15°C) at a relative humidity of 25-45% with fluctuations of no more than 3% (Riss 1993, Vogt-O'Conner 1996). The useful lifespan of magnetic media is about 10-30 years. Originals of magnetic media should be maintained in cold storage with use copies made available for regular access (Riss 1993, Vogt-O'Conner 1996). Spools of tape should be rewound once every six months to a year at playback speed to reduce print-through and the uneven tension in the roll that builds up from ordinary environmental fluctuations. Refer to Henricks (1992b), McWilliams (1979), Riss (1993), van Bogard (1995), and Vogt-O'Conner (1996) for further discussion of tape and equipment for its storage and protection.

Recorded tapes and computer disks should be kept in archival quality boxes and stored vertically, not horizontal or at an angle, to minimize data migration and bleed-through. Protect these items from heat, light, magnetic fields (including electric motors and audio speakers), and air-borne contaminants. Playback heads on tape equipment should be cleaned regularly and demagnetized after every 20 hours of use.

Monitoring the Storage Environment
Environmental monitoring should be done in all collection storage areas. Recording instruments, such as dataloggers or hygrothermographs, are preferred (see *Appendix III* for suppliers). Dataloggers are more cost-effective than hygrothermographs. A comparison of dataloggers for museum use can be found in Arenstein (2002). If recording equipment is not available, an inexpensive thermometer and humidity indicator can be purchased and read two or three times each day. Environmental records should be archived as part of the permanent documentation of the collection. These records are important for evaluating storage conditions, quality of preservation, and rates of deterioration.

THE COLLECTION STORAGE FACILITY

The collection storage room should be located so that the collection care staff have complete control over access to it. It should not have windows. The shelving and floor loading must be adequate to accommodate the weight of the containers (a one-gallon jar of alcohol with specimens may weight more than eight pounds). Moveable shelving (compactors) may be used to maximize space (Fenner 1992). If shelving is constructed more than five shelves high, a safety ladder will be needed for access. Switches should be wired so that lights can be turned on selectively in each aisle so that when one area is being worked in, it is not necessary to illuminate the entire room. Ultraviolet filters should be installed on all UV producing light sources. Suspended light fixtures must be high enough to not interfere with access of containers on the shelving. Shelves should be equipped with lips and restraining bars. Colbert (1961) described a sturdy shelf system using wooden shelves on steel frames. Metal shelves should be made of stainless steel, as no coatings applied to metals (e.g., epoxy, paint, electrostatic powder coating) are sufficiently resistant to the movement of specimen containers.

Safe Storage and Handling of Specimens Preserved in Fluid

The federal government closely regulates the sale and use of ethyl alcohol. A permit must be obtained from the Bureau of Alcohol, Tobacco, and Firearms National Revenue Center to purchase tax-free ethyl alcohol (see *Appendix III*). Isopropyl alcohol may be purchased without a permit.

Denatured Alcohol and Alcohol Additives

Ethyl alcohol that has been denatured may be purchased without a permit. Alcohol is denatured by the addition of chemicals to make it unsuitable for human consumption. Common denaturants include methyl alcohol and aviation fuel (Morrison and Boyd 1973). It is often difficult to determine the denaturant and its concentration; suppliers may change denaturants without notice. All denaturants have the potential to render specimens unfit for future chemical analysis. Denatured alcohol is undesirable as a preservative. Collectors or curators may have added other substances to alcohol solutions in an attempt to enhance the utility of the preservative. The most common additives are glycerin, acetic acid, and formaldehyde. Alcohol solutions with these or other additives should not be used for specimen preservation.

Safety

Two main safety issues are of concern in fluid preserved collections. The first is to ensure that the collection is stored and handled in ways that are safe both for the specimens and for the collection care workers. The second consideration is compliance with fire codes and hazardous chemical regulations.

Safe storage of preserving fluids begins with using containers that provide adequate seals, and keeping the containers on appropriate shelving. The collection storage area must be adequately ventilated. Alcohol and formaldehyde are absorbed through the skin and the mucous membranes of the body, particularly through the lungs (see *Formaldehyde Safety*). Work areas should have bench-top fume collectors positioned so that the fumes are drawn directly from the mouths of containers or from trays of specimens. Open containers of fluid-preserved specimens only in areas with proper ventilation.

A small amount of evaporation of preservative occurs constantly in a fluid collection, either by gas escaping from containers due to changes in temperature and air pressure, or when containers are opened to access specimens. Work areas and storage areas must have sufficient airflow to prevent the accumulation of preservative fumes. Because alcohol is heavier than air, alcohol fumes accumulate at floor level, so the ventilation system must function at floor level. Keep equipment that produces sparks, flames, or heat out of and away from collection storage areas. A strict no-smoking policy must be enforced in the collection storage and work areas. Class A-B-C fire extinguishers should be available in both work and storage areas.

Fire Code and Safety Regulations

When the vapors from flammable or combustible liquids mix with air and come into contact with an ignition source, they can ignite. Fire prevention is directed toward reducing the escape of vapors, diluting the vapors to a safe level, and eliminating ignition sources such as sparks, static electricity, hot surfaces, or flames.

The use and storage of fluid preserved scientific specimens in natural history collections is not addressed directly in the Uniform Fire Code (International Fire Code Institute 1997a) or the Uniform Fire Code Standards (International Fire Code Institute 1997b). The fire code addresses the storage of bulk containers of full-strength (95%) alcohol and the storage of retail liquor (usually 5-15% alcohol). The containers and concentrations of fluid preservative used in collections do not fit either of these categories, so it is necessary to work out an acceptable fire prevention and safety plan with the appropriate local officials. Discuss fire and safety issues with local officials so that they understand that (1) fluid preservatives are diluted for museum use; (2) most preserved specimens are kept in small, well-sealed containers; and (3) the risk of spillage or breakage is very low.

Fluid preservatives may divided into three classes (I, II, and III) and three groups (A, B, and C) based on their *flash point* and *boiling point*. The *flash point* is the lowest temperature at which a liquid releases enough vapors to start burning if ignited. Alcohols are *flammable liquids*; formaldehyde is a *combustible liquid*. A *flammable liquid* is defined as one which has a flash point below 100°F (37.8°C). A *combustible liquid* has a flash point above 100°F (37.8°C). Preservative strength ethyl alcohol (70%) is a Class 1B flammable liquid. Preservative strength isopropyl alcohol (40-55%) is a Class 1C flammable liquid. The flash points and standard preservative strengths for these fluids are indicated in Table 4.

Table 4. Commonly used preservatives.

Names	Chemical formula	Flash point	Vapor density (air = 1.0)	Standard preservative strength
Ethyl alcohol, ethanol, grain alcohol, ETOH	CH_3CH_2OH	70°F in 70% solution	1.59	70%
Isopropyl alcohol, isopropanol, rubbing alcohol	$CH_3CHOHCH_3$	74°F in 55% solution	2.07	40-55%
Formaldehyde, formalin	HCHO	130°F in 37% solution	1.00	3.7% ("10% formalin" contains 3.7% formaldehyde)

Fire code interpretations for fluid-preserved collections may include: removing all sources of ignition from collection storage areas; limiting shelving to no more than seven feet above the floor; requiring explosion-proof fixtures and switches; requiring both heat and smoke detectors; and requiring a sprinkler system. Sprinkler systems in collection storage areas are usually required to have an output of 0.30 gpm/ft^2 (gallons per minute per square foot) over the entire floor area, and 0.21 gpm/ft^2 in laboratory areas. Ventilation requirements are designed to prevent the accumulation of vapors. This usually means airflow of 1 $ft^3/m/ft^2$ (cubic feet per minute per square foot) for collection storage areas and laboratory ventilation of 5 $ft^3/m/ft^2$. Restrictions may be imposed on the amount of preservative stored per room; the storage area may require a reduced temperature (65-70°F is preferred); and access to the storage area may need to be strictly controlled.

Flammable and combustible liquids cannot be stored in basements. The Uniform Fire Code limits storage volumes of Class 1B fluids to 15,000 gallons on the ground floor of a building and 12,000 gallons per floor on upper floors. Shelving must have a lip or guard to prevent individual containers from falling. The Uniform Fire Code contains specific requirements for both metal and wooden shelving systems.

When dispensing flammable or combustible liquids from a storage drum, the pump must be grounded to eliminate static electricity and sparking. Connect the supplying container to the receiving container with a *bonding wire*. Connect a *grounding wire* between the pump and a grounded structure such as a grounded water pipe or metal framework. A *bonding wire* or *grounding wire* is a length of electrical wire with a metal clip on each end for easy attachment to an object or structure. Metal and plastic supply containers, drums, and pumps must be grounded.

All storage and laboratory areas should be equipped with a *spill kit* for safe, efficient clean up of preservative spills. A *spill kit* is a five-gallon, high-density polyethylene (HDPE) bucket with a lid. The contents should include absorbent pads, a small spill boom or dike, Neoprene® gloves, safety glasses, a Tyvek® suit, and plastic bags (see *Appendix III* for suppliers). Clean up preservative spills immediately with the absorbent pads and booms. Enclose the used pads and booms in a plastic bag for disposal.

Laboratory areas should be equipped with eye wash stations and an emergency shower. Eye wash stations should be tested weekly (by flushing water through the nozzle); safety showers should be tested monthly. When working with fluid-preserved specimens or preservative fluids, wear safety glasses and Neoprene® gloves (which are rated for formaldehyde exposure).

THE PREPARATION LABORATORY

The workspace for cataloging, specimen processing, loan preparation and receipt, and related activities is the preparation laboratory. It should be equipped with bench top fume collectors, a sink with hot and cold water, and cabinets and drawers for the storage of supplies and equipment. It should be well ventilated, well lighted, and have UV filters on all ultraviolet producing light sources. A supply of deionized or distilled water should be available for mixing fixative and preservative solutions.

Offices

The offices for collection managers, curators, and researchers should be separate from the preparation laboratory and collection storage areas. Office activities, preparation work, and collection storage activities are rarely compatible. The collection manager's office should be equipped with a good computer and printer, desk, and sufficient file cabinets for working collection records.

Library

Bibliographic and other reference materials for specimen identification, preservation, and management are essential. The library may also house field notes, collection archives, photographic collections, and other relevant items. The library should be separate from the preparation laboratory and collection storage areas. Preparation and collection storage activities are not compatible with a library environment.

Other Requirements

Catalogs should be protected from water, leaks, floods, fire, spilled coffee, and other common museum catastrophes. Catalogs and other important collection records should be backed up by offsite archival microfilm copies (see *Handwritten Catalogs and Electronic Data Storage*). Database records should be backed up regularly and the backup copies stored offsite.

PERSONNEL AND DUTIES

> Such staff as there were [at the British Museum] were overworked and underpaid and often had to take on outside work to support themselves. There were no pensions, so appointees tended to stay at their posts until they died or went mad, which they did with astonishing frequency.
> —Lynn Barber (1980)

Although the number of personnel will depend upon the size of a particular collection and, regrettably, on the finances of the institution housing it, the following job titles and general areas of responsibility are found in many museum situations.

Curator

Historically, the curator was the person who had sole responsibility for the care of the collection, but curators now do little actual collection care (Simmons 1993). A natural history museum curator is usually a scientist who conducts specimen-based research. In most museums, the curator has the ultimate responsibility for the collection, but has minimal involvement in the day-to-day management of the collection (Laub 1985). In addition to research responsibilities, the curator approves all requests to use the collection, sets the taxonomic standards for the collection, is responsible for collection growth, and works with the collection manager to establish and implement collection policies.

A curator should have a terminal research degree, an expert knowledge of systematic herpetology, and be actively involved in specimen-based research.

Collection Manager

The collection manager manages the collection and performs the collection care duties formerly assigned to the curator (Cato 1991, Ford and Simmons 1997, Simmons 1993). The collection manager is responsible for the care, conservation, and management of the collection and collection records, and access to specimens and data. The collection manager, with the curator, develops and implements collection policies. The collection manager is responsible for monitoring the collection storage environment, approving and obtaining collection care materials and chemicals, supervising collection use, and supervising other collection care workers. The collection manager makes regular visual inspections of the collection storage areas to detect preservative loss, presence of pests, and disorder in the collection, and records and evaluates collection activities.

A collection manager should be a professional with an advanced degree in museum studies (with particular emphasis on collection management and conservation), an understanding of systematics, and a familiarity with the herpetological literature. Useful skills and knowledge areas for collection managers include technical and grant writing, materials science, mechanical ability, database use, and information management.

Curatorial Assistants

Curatorial assistants do much of the hands-on work of collection care, working under the direction of the collection manager. Curatorial assistants label containers, tie tags, top off containers of preservative, shelve containers, file documents, pack and unpack specimens, and perform assorted other tasks.

COLLECTION MANAGEMENT

> A museum has a responsibility to provide reasonable care of the objects entrusted to it...As a practical matter, this means that...museum policies and procedures afford prudent care and protection for all museum objects in light of existing circumstances.
> —M.C. Malaro (1998)

Collection Management Policy

The purpose of a collection management policy is to ensure that the institution meets its legal and ethical obligations to care for the collections in a professional manner. The policy should define collection care standards, areas of responsibility and establish collection documentation requirements. Excellent guidelines for collection management policies are provided by Cato and Williams (1993) and Malaro (1998).

The collection management policy should address requirements and standards for accession and deaccession of specimens, documentation, cataloging, tagging and labeling, standards for the care of collections, access to the collection and use of the collection (including loans, gifts, and exchanges), access to and use of collection information, collection evaluation, and collection growth. These topics are addressed in the appropriate sections below. A guide to locating additional information on collection management policies and procedures is provided in Table 5.

The entire collection management policy, as well as collection care procedures, should be reviewed periodically by the curator and the collection manager.

Accession of Specimens

Accessioning means adding a specimen to the collection. It is the formal process by which the museum takes legal possession of the specimen, and encompasses the entire process from receipt of material in the museum through its preparation for cataloging (Buck and Gilmore 1998, Malaro 1998). It includes the preparation and archiving of appropriate documentation. At the time of accession, the specimen data should be checked for completeness and accuracy to avoid delay during the cataloging process.

An *accession* is one or more objects (or specimens) received from a single source, at one time. The *accession record* contains the essential documents and information describing the transaction through which the museum acquired the specimens. It is part of the permanent documentation of the museum. The accession record should show the date of receipt, date of accession, collector's or donor's name and address, and a description sufficient to identify the specimens. Each accession is assigned a sequential accession number by the institution. This number should be used to cross-reference all collection documentation and to label the specimens until they are cataloged. The accession number should be listed in the catalog entry for the specimen.

Table 5. Resources for developing collection management policies and procedures.

Subject	Section of this publication	Additional references
Field preservation and documentation	Preservation of Specimens	Casas-Andreu et al. 1991, Garrett 1989, Scrocchi and Kretzschmar 1996
Conservation of the collection	Conservation of Herpetological Collections	Cato and Williams 1993, National Park Service 1996, Simmons 1995, SPNHC 1994
Policy (general)	Museum Collections; Collection Management Policy	Buck and Gilmore 1998, Cato and Williams 1993, Malaro 1998, SPNHC 1994
Acquisition policy	The Accession Process: Field Collecting; Guidelines for Collection Growth	Buck and Gilmore 1998, Cato and Williams 1993, Fritts 1976, Lee et al. 1982, Malaro 1998, Merritt and Lidgard 2000
Accession policy	The Accession Process	Buck and Gilmore 1998, Cato and Williams 1993, Malaro 1998
Loan policy	Loans, Gifts and Exchanges	Buck and Gilmore 1998, Malaro 1998, Merritt 1992
Collection use policy	Categories of Loans; Evaluation of Collections	Cato and Williams 1993, Malaro 1998
Information management	Cataloging Specimens	Buck and Gilmore 1998, Cato and Williams 1993
Destructive/Consumptive sampling policy	Loan Policy	Cato 1993, Cato and Schmidly 1991, Cato and Williams 1993
Deaccession policy	Cataloging Specimens	Cato and Williams 1993, Malaro 1998
Standards of collection care	Collection Management; Personnel and Duties; The Collection Storage Facility	National Park Service 1996, Simmons 1995, SPNHC 1994
Collection Evaluation	Annual Reports of Collection Management Activities; Evaluation of Collections	McGinley 1993; Merritt and Lidgard 2000; Price and Fitzgerald 1996; Waller 1995; Williams et al. 1996

Accessions must be relevant to the purposes and priorities of the collection; the museum must be able to provide proper storage and care of all specimens it accessions, without compromising its ability to store and care for what it already has. All accessioned specimens must be obtained legally and they must be properly documented and labeled. The responsibility for the registration of accessions may rest with the collection manager, registrar, curator, or other administrator, depending upon the institutional organization.

Guidelines for Accessioning Decisions

1. Do the specimens fall within the categories identified as important to collection growth?
2. Are the specimens appropriate for the collection? Could they be of more use in another institution?
3. Are the specimens well preserved?
4. Are the field notes and other documentation in good order?
5. Are there originals or copies of the proper permits for collecting, exporting, and importing the specimens?
6. Has a clear understanding been reached with the donor regarding the terms of deposit of the specimens (see *Deed of Gift/Deed of Transfer*)?
7. If specimens come from a zoo or other live-animal facility, have proper necropsy protocols been followed (Jacobson 1978)? Were the specimens exposed to toxins, infectious agents, or radiation?

Documentation of Collections

Field Notes

The field notes consist of the field journal and the field catalog prepared by the collector at the time the specimens were collected in the field (see *Field Notes*). The field notes are extremely valuable. These documents should be archived in the same institution as the specimens, and cross-referenced to include the museum catalog numbers assigned to the specimens. If it is not possible to obtain the original field notes, at least obtain an archival quality copy of them. Refer to Jordan (1993) for guidelines for preservation photocopying.

Legal Documents

All incoming specimens must be obtained legally (see *Permits*). Originals or archival quality copies of all permits, declarations, export and import forms and permits, other permissions to obtain, possess, and transport the specimens, correspondence, and deed of gift or deed of transfer are part of the accession record.

Images

Photographs, negatives, color transparencies, and digitized images should be referenced with the accession number and, where appropriate, with the museum catalog number of the individual specimen(s) depicted. Each image should be labeled with a separate, sequential number. Store prints, negatives, and transparencies in appropriate archival quality materials (see *The Storage Environment for Herpetological Collections*). Keep a proof sheet of negatives on file to assist in locating the images.

Recordings

Each recording should be labeled with a separate, sequential number. Audio and video recordings on tape have a very limited life. Tape must be handled with great care. The only way to prolong the useful life of tape recordings is to digitize the recorded information and store it in a medium acceptable for longer-term storage (e.g., on compact disc).

The Accession Process

When specimens are received, they should be unpacked, properly housed, and labeled pending accession.

Step 1. Receiving the Specimens

Unpack incoming collections with care, watching for loose specimens, ruptured containers, information written on containers, field notes stuck between the packed specimens, or slips of paper containing data tucked in with specimens but not attached to them. Some collectors can be quite creative when they pack specimens. Check all specimens against the packing list provided. If no such list was provided, make a complete list of specimens received and include this in the accession documents. Provisionally mark each container with the name of the source (e.g., donor or collector of the specimens) until an accession number becomes available.

Step 2. Complete the Accession Checklist

Complete an *accession checklist* for the specimens. The accession checklist is a standardized form that is used for each potential accession to help ensure that all necessary documentation is in place for the museum to assume ownership of the collection. An example of an accession checklist is shown in Figure 7. The accession checklist should include:

- *Accession number* (to be filled in later when it is assigned by the museum).
- *Catalog numbers* when they are assigned to the specimens.
- *Collectors*. Full names of all collectors who contributed to the collection.
- *Date* the specimens were received by the museum.
- *Description* of the collection, including the number of individual specimens.
- *List of permits, field notes, correspondence, and other documentation* that accompanies the collection.
- *Name and signature* of the person responsible for completing the accession checklist.
- *Source*. Name, address, telephone, and email address of the collector, donor, seller, or museum donating the specimens.

Step 3. Assemble the Accession File

Assemble the documents that make up the Accession File. These include the:

- Accession Checklist.
- Correspondence relating to the collection, transport, and ownership of the specimens.
- Deed of Gift/Deed of Transfer.
- Field Notes (these are not usually included in the file itself, but their location in the museum should be noted).
- Permits.
- Other documents pertaining to the accession.

Step 4. Obtain an Accession Number

Submit the appropriate documentation to obtain an accession number (see *Accession of Specimens*). This step establishes the legality of the accession, and is the time at which the museum takes legal ownership of the specimens. Label all documents and specimen containers with the accession number as soon as the number is available.

Deed of Gift/Deed of Transfer

Each donating institution or individual must sign a Deed of Gift or Deed of Transfer document (Malaro 1998). This document (1) is a statement of the good-faith effort of the institution or individual to demonstrate that the accession was obtained legally; and (2) establishes the clear ownership of the museum of the accession. An example of a Deed of Transfer is shown in Figure 8.

Selection of a Preservative

Since the mid-1600s, when the preservative properties of spirit of wine were discovered, the overwhelming favorite fluid preservative has been ethyl alcohol, usually in a 60-75% concentration. The second most common preservative (though far less common than ethyl alcohol) is isopropyl alcohol, which is less expensive and easier to obtain. Isopropyl alcohol was not used as a preservative until sometime after 1920 when commercial production of it began (Hatch 1961); thus, we have fewer than 80 years of experience with it as a preservative. The third common preservative is formaldehyde, which was not used as a preservative until sometime after 1893 (Simmons 1995). Methyl alcohol (wood alcohol) is unsuitable for use as a preservative.

The biggest drawbacks to ethyl alcohol are its expense, the necessity of obtaining a Federal permit to purchase it tax-free, and the potential fire hazard it poses. The arguments in favor of using isopropyl alcohol are that it is less expensive and easier to obtain (a Federal permit is not required), and that it leaves the specimens more flexible (Walker et al. 1995). However, there are many more arguments against its use. Isopropyl alcohol poses a fire hazard, it is twice as toxic as ethyl alcohol, and many people find its odor objectionable. The greater flexibility of the specimens may mask warning signs of deterioration. Isopropyl

Figure 7. Accession Checklist.

Accession Checklist

The following checklist is to be completed before any specimen or object may be accessioned by the Natural History Museum. Check boxes ONLY if the required documents are in hand.

RECEIPT OF SPECIMENS PREVIOUSLY CATALOGED IN ANOTHER MUSEUM

(by donation, exchange, gift, abandonment, or purchase)	Yes	No	Not Applicable
Transmittal form or letter from appropriate authority at the institution of origin	☐	☐	☐
Signed Deed of Transfer form	☐	☐	☐
Export permit (if from a non-US institution)	☐	☐	☐
Import permit (if from a non-US institution)	☐	☐	☐
CITES permits if transaction involves CITES-listed specimens	☐	☐	☐
APHIS certification	☐	☐	☐
Other:	☐	☐	☐

RECEIPT OF SPECIMENS NOT PREVIOUSLY CATALOGED IN ANOTHER MUSEUM

(field work, gift, exchange, purchase, donation, bequest, or contract)	Yes	No	Not Applicable
Original or copy of collecting permit(s)	☐	☐	☐
Original or copy of export permit (if from a non-US institution)	☐	☐	☐
Signed Deed of Transfer form	☐	☐	☐
Current US Fish and Wildlife Service permit on file	☐	☐	☐
Copy of 3-177 form (if from a non-US institution)	☐	☐	☐
CITES permits if transaction involves CITES-listed specimens	☐	☐	☐
Antarctic Conservation Act permit	☐	☐	☐
Bald Eagle Protection Act permit	☐	☐	☐
Bureau of Land Management permit	☐	☐	☐
Controlled Substances Act	☐	☐	☐
Feather Import Quota	☐	☐	☐
Federal Noxious Weed Act	☐	☐	☐
Fur Seal Act	☐	☐	☐
Marine Mammal Protection Act permit	☐	☐	☐
Migratory Bird Treaty Act permit	☐	☐	☐
Plant Pest Act	☐	☐	☐
Plant Quarantine Act	☐	☐	☐
State Collecting permit	☐	☐	☐
US Fish and Wildlife Salvage permit	☐	☐	☐
APHIS certification	☐	☐	☐
Original or copy of field notes for specimens in the accession	☐	☐	☐
Originals or copies of any correspondence relating to this accession	☐	☐	☐
Other:	☐	☐	☐

Figure 7. Accession Checklist. (continued)

I hereby certify that to the best of my knowledge the above information is correct and accurate and that the specimens and/or objects comprising this accession were obtained legally.

Signature:____________________ Title:__________ Date:________

This accession is approved for acceptance and cataloging by the Natural History Museum of the University of Kansas.

Signature:____________________ Title:__________ Date:________

Accession Number: ____________________

Catalog numbers assigned to accession:

List of support documents:

Figure 8. Deed of Transfer.

NATURAL HISTORY MUSEUM
Deed of Transfer

Date:____________________

Received from:

Name:____

Address:____________________________________

____

____

Telephone:_________________________________

Description of Accession:

AGREEMENT

I hereby acknowledge that I have read the terms of acceptance of this accession, and that to the best of my knowledge, the specimens and/or objects comprising this accession were obtained legally.

Signature of Donor or

Agent:____

Date:_____________________________________

alcohol does not have the 350+ year record of use in museums that ethyl alcohol does. The use of isopropyl alcohol as a preservative makes specimens unsuitable for most types of histological preparation (Jones and Owen 1987). It is difficult to measure the density of isopropyl alcohol solutions, particularly with a hydrometer, as its density is close to that of water. In low concentrations (below 45%), isopropyl may promote a rapid clearing of tissues. Isopropyl alcohol is difficult to mix with water and prone to layering in containers (Fink et al. 1979). In long-term storage, it has been shown to soften bone (Steedman 1976). While perhaps an argument might be made to continue using isopropyl alcohol for fish collections already in it (Walker et al. 1995), the argument does not justify using a preservative other than ethyl alcohol to preserve herpetological collections.

Just because most fluid preserved collections use ethyl alcohol does not mean that it is the best preservative to use. However, no data indicate that any other preservative is superior, or even the equal of ethyl alcohol (Simmons 1995). Some alternate preservative formulas have been proposed for specific functions, such as preserving colors of specimens (see *Color Preservation*), or making specimens useful for specific histological techniques. A few substances, such as phenoxetol, were in use for a decade or more before preservation problems were detected (Crimmen 1989). In the absence of carefully controlled, long-term or accelerated aging studies of other preservative solutions to demonstrate their superiority, fluid preserved specimens should continue to be kept in ethyl alcohol.

Transfer of Specimens from Fixative to Preservative

It has long been customary to remove specimens from the field fixative, rinse or soak them in water in open containers (sometimes for several days), and then place them directly in storage-strength alcohol preservative (Simmons 1995). There are several problems with this procedure. Damage may result from soaking specimens too long in water. Once formaldehyde is replaced with water, enzymatic activity in the tissues may resume (Taylor 1981a) and the water may reverse the chemical crosslinks, permitting autolysis to occur. Specimens will swell as they absorb water during rinsing or soaking and shrink once they are placed in an alcohol preservative solution. Some workers have suggested transferring specimens directly from the formalin fixative solution to the alcohol preservative (Fink et al. 1979). This procedure has several drawbacks, including severe osmotic shock in going from nearly 100% water to 30% water, and the quantity of formaldehyde that is released into the alcohol preservative. Formaldehyde in the preservative will drive the acidification of the alcohol solution, which will decalcify specimens and cause more severe color changes, as well as pose serious health risks for people working with the specimens.

Based on the studies available on shrinkage, swelling, and other effects on specimens during transfer (e.g., Jones and Owen 1987, Lafromboise et al. 1993; see discussion in Simmons 1995), and considering the effects of osmotic pressure changes on tissues (Steedman 1976), a more gradual transfer of specimens from a solution of near 100% water (e.g., "10% formalin") to 30% water (70% ETOH) is recommended. Because osmotic pressure rises steadily with ethanol concentrations below about 75%, it has been suggested that approximately equal concentration increments are the most appropriate for stepping specimens up to higher ethanol concentrations (Waller and Strang 1996). Following the protocol of Laframboise et al. (1993), I recommend using steps of approximately 20% to stage specimens from 10% formalin solutions to 70% ethyl alcohol (Figure 9).

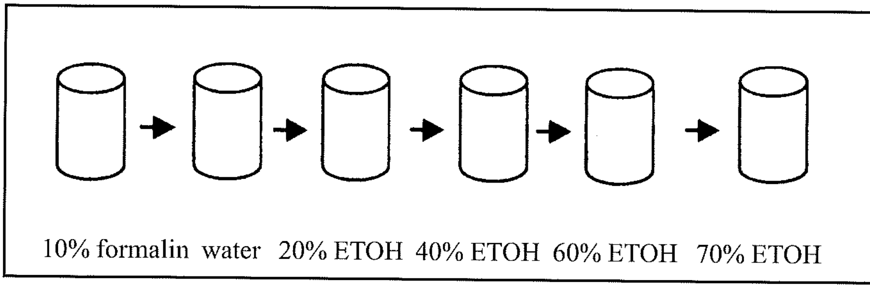


Figure 9. Transfer of specimens from field fixative to museum

Trace Amounts of Formaldehyde in Preservative Solutions

It is difficult (and probably undesirable) to completely remove the formaldehyde fixative from specimens fixed in 10% formalin by washing. As a result, some trace amounts of formaldehyde will remain in the alcohol preservative. One study found these amounts to average 0.07% in one collection (Simmons and Waller 1994). Trace amounts of formaldehyde in preservative solutions can be detected by using formaldehyde test strips. Test strips can be made by following the instructions in Waller and McAlister (1986), or a commercial formaldehyde test kit may be purchased (see *Appendix III*). Trace amounts of formaldehyde are usually insufficient to affect the quality of the preservative; however, in some cases formaldehyde and other sources of acid (e.g., label paper) have been shown to acidify alcohol solutions (Andrei and Genoways 1999, Van Guelpen 1999).

Transfer of Specimens between Alcohols

Specimens should not be changed from one type of alcohol to another, especially specimens on loan from other institutions. Changing a specimen from one type of alcohol to another, particularly from isopropanol to ethanol, may cause significant shrinkage and other damage (Jones and Owen 1987) and significantly alter the body proportions of the specimens (see review in Simmons 1993). If it is necessary to change specimens from one alcohol preservative to another, follow the protocol of Laframboise et al. (1993).

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 collection. The *catalog number* is not assigned to a specimen until it has been through the complete accession process (see *Accession of Specimens*), 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.

Cross-cataloging is the sorting of specimen data into other categories (e.g., by geography or by taxa). Historically, this procedure required the creation of new file cards or other hard-copy specimen records, but cross-cataloging is most efficiently done with an electronic database.

Hand-Written Catalogs and Electronic Data Storage

For hundreds of years, the tradition in natural history museums has been to maintain a hand-written, sequential catalog (or register) that lists each individual specimen by number, along with pertinent data. It is very difficult to work with the information in this format; thus, most institutions had card files or loose-leaf notebooks in which the information was rearranged by categories (see *Cross-Cataloging*). Now that specimen data may be more easily and efficiently entered into an electronic database (see *Electronic Databases*), many institutions have failed to maintain their hand-written catalogs, usually citing the presumed expense of writing information by hand.

Although an electronic database is clearly superior for data manipulation, electronic data are not permanent. It is important to distinguish between an *archival copy* of catalog data and a *backup copy*. An archival copy is a permanent document, using the best materials available for long-term storage. A backup copy of an electronic database is a safeguard used to rebuild the data should an accident occur. A backup copy may or may not be in a permanent format. A writeable CD is an excellent backup format. A hand-written catalog or a microfilm copy are the best archival copies of a database.

No system of recording data is as permanent as using an archival black ink on acid-free 100% rag paper. If cared for properly, a hand-written catalog on good paper will last at least 400 years, probably much longer, and still be legible to a reader. No form of electronic data storage or machine printing will last this long (Simmons 1998). The most permanent means of storing digitized data at present is on compact disk (CD). CDs are made of a polycarbonate plastic that is more susceptible to damage from heat, moisture, light (which causes the plastic to yellow), abrasion, and chemical exposure (e.g., to compounds containing benzene rings, such as naphthalene or paradichlorobenzene) than is paper. A CD also requires a functioning CD reader for access to the data. It is often assumed that digitally stored data can be accurately transferred from one generation of digital storage to another (e.g., from IBM punch cards to floppy disks). However, the short history of museum computerization has demonstrated that software and hardware limitations always result in some loss or corruption of data. Electronic data in a digital format is not archival.

Abandoning the hand-written catalog because it contains the same information as the electronic database is foolishness. The hand-written listing of specimens added to the collection is a legal document that records the sequence in which objects were added to the collection (Baron 1991). Unlike electronic databases, records cannot be altered or eliminated from a hand-written ledger without leaving behind evidence of the modification. Electronic databases require regular backup, regular upgrades, periodic hardware upgrades, and do not provide the permanent association of specimen and catalog number that a hand-written catalog provides. Few electronic databases preserve the specimen record as originally written. Often, this information is critical in evaluating a specimen record years after it is entered in the database. Hand-written catalogs are expensive to buy and take time to write in, but they remain the only permanent, long-term means of meeting the museum's legal obligation to preserve vital collection data.

The hand-written catalog should contain pen-ruled sheets of acid free, 100% rag paper, folded in signatures and permanently bound in hard covers. Because the hand-written catalog is the primary listing of specimens in the collection, it must be treated as an archival document and carefully protected. A microfilm backup copy should be stored off-site.

Hand-Written Catalog Entries

Information in the hand-written catalog should be neatly printed in an archivally stable black ink (see Williams and Hawks 1986 and Williams and Hawks 1988 for comparisons of inks). Never use disposable pens, as these inks contain additives that may cause deterioration of the image or the paper over time. Minor mistakes may be neatly erased with a clean, high quality ink eraser. Larger errors should be crossed out with one line, the correction written in 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. Whether making entries in a hand-written catalog or into an electronic database, the following general procedure should be followed.

Arrangement of Material for Cataloging

The specimens to be cataloged should be arranged so that within a given locality, all individuals of each species can be numbered sequentially. This method enables the cataloging process to proceed much faster and more efficiently by maximizing amount of data that can be duplicated, saves space by allowing series to be printed on container labels (Williams et al. 1977), and makes it easier to locate individual specimens of a geographic or taxonomic unit.

The following information 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. Refer to Riemer (1954), Axtell (1965), and Hutchinson (1964) for a discussion of localities and standardized formats.
Coordinates. Include coordinates whenever possible. The format of map coordinates will depend on how they were obtained (e.g., from a GPS unit in the field, or calculated from a map). The record should indicate the format of the coordinate data (e.g., minutes and seconds, tenths, or hundredths of units).

The traditional catalog entry in standard format is written as follows:

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

Example:

> Brazil: Amazonas: Castanho: approximately 40 km S Manaus, km 12 on road to Autazes, 03° 37' 10.4" S, 59° 86' 78.4" W, 50 m

Date. Enter dates as the numerical day, the first three letters of the month, and the full numerical year, as in 12 Feb 1809. Systems using all numerals are too easily misunderstood, because the convention determining whether day or month comes first varies from country to country.
Scientific Name. No universally agreed upon lists of scientific names for amphibians and reptiles exists. Personnel at each institution must determine which reference lists they will follow. Consistent use of a standard taxonomy is recommended; the curator and collection manager must make judgements concerning names they will consider valid. Two useful on-line taxonomic sources are listed in Appendix III.
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. Museum names may be abbreviated by using the standard symbolic code of the institution (Leviton et al. 1985).
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 larvae or a 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, a specimen in Bouin's solution, a specimen in glycerin), or if the specimens must be stored under special conditions (e.g., dry).
Type Status. Note if the specimen is a holotype or paratype of a valid or synonymized taxon.
Developmental Stage of Specimen. The default is a sexually mature adult. Note if the specimen is a juvenile, neonate, egg, larvae, etc.
Accession Number. Record the accession number assigned to the specimen.
Remarks

- Record any reference(s) to any specimen documentation such as images, recordings, field notes or other pertinent documentation.
- Note if the specimen was maintained live in captivity before it was processed, if it was captive born, or captive raised.
- Record other information as appropriate.

Tagging Specimens

Once a specimen has been assigned a catalog number, attach the numbered tag to the specimen (see *Specimen Tags*).

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, caecilians, limbless lizards and salamanders, and amphisbaenids, 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 too 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, place the individual specimen and the tag (with about an inch of string attached) in a shell vial filled with the appropriate fluid preservative. Position the specimen and tag in the vial so that the tag can be read from the outside. Close the vial with a secure 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. For catalog numbers assigned to larvae (whether one individual or a lot), place the tag (with about an inch of string attached) in a shell vial with the specimen(s), and fill it with the appropriate fluid preservative. Position the tag so that it can be read from the outside. Close the vial with a secure 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.

7. Never remove field tags or previous museum tags from a specimen unless the tags are deteriorating. Deteriorating tags should be replaced with substitutes, and the originals archived in mylar enclosures (Kishinami 1989, 1992).

Electronic Databases

Although an electronic database is not a replacement for a hand-written catalog (see *Hand-Written Catalogs and Electronic Data Storage*), it is extremely useful. An electronic database is not merely a digitized version of the collection catalog—it is a collection management tool. Electronic databases can handle more information faster and with greater accuracy than manual systems. Much more information can (and should be) be included in an electronic database than in a hand-written catalog. For example, with an electronic database, field notes can be digitized and linked to specimen data records, enabling the collection user to see the page of notes as written by the collector, rather than see an edited version of what the notes contain.

An electronic database for collection management needs to meet the following minimum requirements:

1. Have the ability to handle the size of the collection plus allow for growth.
2. Be an easily accessible relational database.
3. Allow data input without using codes or abbreviations.
4. Be fully searchable. For example, one should be able to sort data by various combinations of taxonomy, geography, type status, preservation type, collector, date of collection, accession number, taxonomic level, or ancillary information (e.g., images or recordings).
5. Have a good report writing function for the production of written reports, labels, loan forms and other documents, as well as the electronic production and export of reports.
6. Allow correlation of support documentation (such as audio recordings, visual images, and field notes) with the cataloged specimens.
7. Accommodate information such as references to the citation of specimens by catalog numbers in the literature.
8. Be reasonably easy to learn to use.
9. Be well supported with good technical help available via a toll-free help line.

The use of an electronic database obligates the museum to a perpetual expense in data transfer as software is updated, and technology transfer as each new generation of computers appears. Multiple backups will reduce the risk of data loss during data transfer. Software upgrades must be done in a timely fashion, or you may find yourself in the position of having software you can no longer upgrade. Database maintenance should not interfere with the collection managers' collection care duties. The primary focus of the collection manager should be care of the collection, using the database as just one of many tools for that purpose.

An electronic database can be used to track collection growth and use. With the ability to process much more information about individual specimens than in a manual system, preparation techniques can be associated with specimens and evaluated for their effect on specimen preservation and rates of deterioration.

Electronic databases make it possible for collection records to be available online. Such systems need to be designed with a good firewall to protect specimen data from hackers and viruses, and with systems to limit certain sensitive data (e.g., exact localities of commercially valuable species).

Specimen Tags

Historically, specimen tags have been almost as varied as the specimens to which they were attached (Hawks and Williams 1986). They have been made of paper, wood, metal, plastic, and cloth. Tags have been inscribed with everything from simple numbers to complete collecting and locality data. For use with fluid preserved specimens, tags should be kept small and simple.

1. Use a small tag format to reduce the possibility of damage to both tag and specimen.
2. Choose a fluid-resistant material that is durable but does not have sharp edges or corners that could damage specimens.
3. Do not use materials that might damage the specimen as they deteriorate (e.g., polyvinylchloride plastic).
4. The tag should have the museum name, acronym, or symbolic code (Leviton et al. 1985), plus the catalog number on it. The catalog number should be stamped on the tags so that if the ink is lost, the number can still be read in a raking light.

The best tag material is white, 100% sturdy cotton stock, with a pH of 6.5 to 7.0 (Hawks and Williams 1986), machine printed in indelible carbon black printing ink, with the sequential catalog number on one side and the museum name, acronym, or symbolic code on the other side. A small hole should be machine punched in one end of the tag to accommodate a soft thread for attaching the tag to the specimen.

An alternative material for tags is spunbonded polyethylene (sold under such names as Tyvek® or Polypaper®). Tags made of this material may have sharp corners—if so, trim the corners so that they are rounded.

Do not use plastic tag stock made of unstable materials, plastics with added color, or materials with adhesive backing (e.g., Dymo® labels) because all of these materials will contaminate preservative solutions.

Stringing Specimen Tags

To facilitate stringing tags, a tag vice is useful. This is made from two lengths of wood, 12-15 inches long, which can be clamped together by two bolts. A series of tags is clamped in the vice with the holes outward for stringing. This holds the tags rigid and frees both hands for stringing.

Tags should be strung with soft cotton or linen thread so that they are ready to attach to the specimen (I prefer a 100% mercerized soft crochet thread without dye).

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 should be printed on fluid-resistant, durable stock. The preferred materials for container labels are spunbonded polyethylene (sold under such names as Tyvek® or Polypaper®; see Gisbert et al. 1990) or white, 100% cotton stock, with a pH of 6.5 to 7.0 (Hawks and Williams 1986).

A popular label stock used in fluid collections, Resistal® paper, is acidic and should not be used. It is fluid resistant due to a melamine ($C_3H_6N_6$) coating, which causes the paper to acidify preservative solutions (Andrei and Genoways 1999, Van Guelpen 1999). Descriptions of a variety of materials and production methods for labels for fluid preserved collections may be found in Snyder (1999).

For long-lasting labels in fluid, the information on the label should be hand-written in indelible ink with a technical pen, or typed with a black carbon ribbon. Erasable Selectric® correcting typewriter ribbons and laser printers do not form a sufficiently strong bond between the ink and the paper. Some combinations of paper and laser printing will hold up fairly well in alcohol solutions, but none is long lasting. Test each combination of paper and ink before using it in the collection. The most long-lasting labels for fluid specimens are those produced by thermal printers that heat bond a carbon powder to a spunbonded polyethylene stock to create a nearly indestructible label (see *Appendix III*).

Combining Specimens in Containers

To save space and container costs, the tradition in herpetology collections is to combine reasonable quantities of specimens in containers. The drawbacks to this practice include the possibility that smaller or fragile specimens may be damaged by larger specimens or by crowding in the container, and that more specimens must be handled to locate the specimen that is needed. The most serious drawback is that when a specimen sits in a container of alcohol, lipids and proteins leach out of the specimen into the solvent, making the surrounding preservative part of the specimen, too (Von Endt 1994). Mixing specimens in containers means mixing the extracted constituents in the fluid.

When combining specimens in containers, put like-with-like. Do not mix different species in a jar, do not mix specimens from different geographical areas in the same container, and do not put too many specimens in one container. When allocating specimens to containers, maintain a ratio of at least twice the volume of liquid as specimens (Zweifel 1966).

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 in the collection.
7. *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 AND CLOSURES

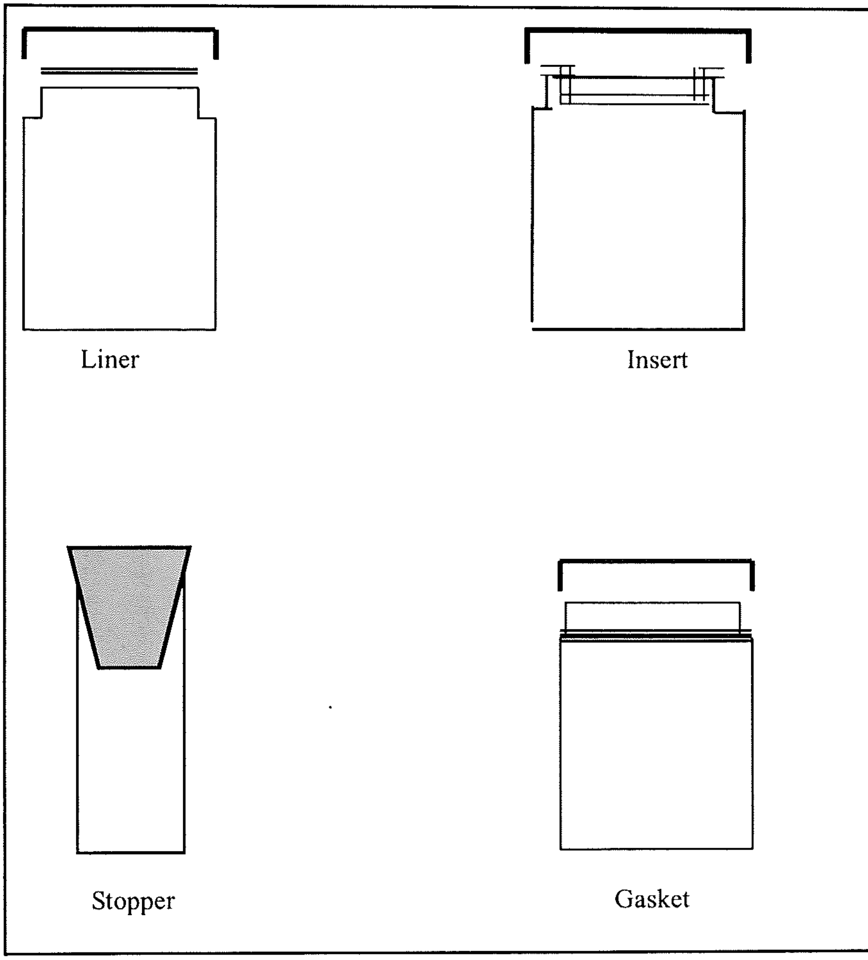
A *container* is a receptacle that holds a specimen and the preservative fluid (see Simmons 1995 and 1999b for more discussion). Containers are usually jars, buckets, or tanks. A bottle has a much greater constriction of the neck and mouth than does a jar.

A *closure* (e.g., a lid and gasket) is the means of sealing the mouth of the container. Closures are either lids or stoppers (Figure 10). A *lid* fits on top of a container, or over the top of a container opening. A *stopper* fits inside a container opening.

The ideal container for fluid preserved specimens is leak-proof (to prevent the loss of preservative), light proof and impervious to oxygen (to protect the specimen), allows for inspection of the specimen without breaking the closure seal, and is made of a non-corrosive, non-reactive material. There are no ideal containers.

General container types are summarized in Table 6. The best containers currently on the market are the alcohol-resistant, precision-ground glass-stoppered museum jars made of borosilicate glass (Clark 1992, 1993). Considering price, availability, and serviceability, the most suitable containers on the market are

Figure 10. Closures and seals.



clear flint glass jars, with flexible polypropylene threaded lids, with a liner or insert (Suzumoto 1992b).

Glass Containers

Most fluid preserved specimens are housed in glass jars. Most glass (with the exception of borosilicate glass) is susceptible to deterioration from alcohol and other preservatives. Older museum jars often look dirty or exhibit iridescence when dry, but when wet appear normal. This is the result of glass deterioration. The deterioration is a slow process, but over time the glass becomes fragile and the deterioration products contaminate the preservative fluid and specimens. Glass deterioration may be accelerated by repeated wetting and drying of the glassware, by exposure to high relative humidity, or by storage at warm temperatures (Matson 1949, Owens and Emanuel 1942, Stockdale and Tooley 1950).

Table 6. General container types.

Type of container	Relative Cost	Availability	Quality of Seal	Longevity of seal	Rating for use
Glass jar with glass stopper	Very high	Poor	Excellent (new jars only)	Life of container	Excellent
Glass jar with glass lid and wire bail	High	Poor	Fair to good	< 10 years	Good
Glass jar with thermoplastic (polypropylene) threaded lid	Medium	Very good	Very good	>20 years	Very good
Glass jar with threaded metal lid	Medium	Good	Fair	< 5 years	Poor
Glass jar with snap-on thermoplastic (polyethylene) lid	Medium	Poor	Fair	5-10 years	Poor
Glass jar with thermoset (phenol, melamine, Bakelite) threaded lid	Medium	Good	Fair	< 5 years	Poor
Glass vial with compressible stopper	Low	Good	Fair	< 10 years	Poor
Polyethylene terephthalate (PET) container with polypropylene closure	Low	Fair	Unknown	Unknown	Unknown
Polycarbonate container	Medium	Fair	Unknown	Unknown	Unknown (closure material unknown)
Stainless steel tank	Very high	Fair	Fair to good	<10 years	Fair
High-density polyethylene bucket or barrel	Low	Good	Good	>15 years	Good

Older glassware should be monitored closely for signs of deteriation.

Closures

There are five basic types of closures used for containers of fluid preserved specimens.

1. Jars with compressible stoppers.
2. Jars with non-compressible stoppers.
3. Jars with lids held in place by a wire bail or clamp.
4. Jars with snap-on plastic lids.
5. Jars with threaded lids.

Closures are often equipped with a liner, a gasket, or an insert to improve the seal (Figure 10). A *liner* is a flat disk that fits inside the lid, usually made of polyethylene (Palmer 1974) or Teflon®. A *gasket* is a compressible material formed in a shape to fit around the rim of the container or the lid. Gaskets may be made of natural rubber (which is usually red or orange); buna-n; buna-n duro (a 50/50 acrylonitrile-butadinene copolyer); or EPDM (ethylene propylene diene ter-polymer). An *insert* is a polyethylene device made to fit into the mouth of the container so that it is held in place when the closure is applied.

Compressible Stoppers

Stoppers may be made of cork, rubber, or a synthetic material (the most common synthetic stopper material is neoprene). All compressible stoppers lose their elasticity and become brittle as they age, and from contact with the preservative. When the stopper fails, evaporation occurs, and the preservative is contaminated by the components of the stopper that leach into it. The result is usually discolored specimens and labels (Nizinski 1987). Cork stoppers contaminate the preservative with tannins and pigments, and crack with age (Galigher and Kozloff 1971, Levi 1966).

Non-compressible Stoppers

Non-compressible stoppers are usually made of glass. These are simple closures (no gasket), but may be sealed with petroleum jelly or a cement. The old style glass stoppers were individually ground to fit a particular jar and were difficult to remove. The newest jars on the market have interchangeable, precision-ground borosilicate glass stoppers with an excellent seal (Clark 1992, 1993). Although extremely expensive to purchase, they may be cost effective in a large collection where containers are not opened frequently (Clark 1992, 1993). They are very useful for exhibition purposes.

Lids Held in Place by a Clamp or Bail

Glass lids are held fast to the jars by pressure from a wire bail or wire clamp, with a compressible gasket to complete the seal. The gaskets leach chemicals into the preservative, contaminating and discoloring it. Over time, the gaskets loose their elasticity and begin to crack, losing their ability to maintain a seal against evaporation. Gaskets made of synthetic material are more durable than those made of natural rubber. See *Appendix III* for suppliers of synthetic gaskets.

Snap-On Lids

Jars with snap-on polyethylene lids are called *Copenhagen jars* or *Danish jars* in Europe. These closures crack and fail due to the degradation of the plastic from age or from exposure to ultraviolet light (e.g., from overhead fluorescent light sources).

Threaded Lids

Threaded (screw-on) jar lids may be made of metal or plastic. Metal lids are prone to oxidation, and are to be avoided. Rigid thermoset lids are inclined to back-off or crack with fluctuations in temperature. The rigid black lids are usually Bakelite. Bakelite is a phenol-formaldehyde polymer that is prone to embrittlement, particularly in the presence of formaldehyde vapors. More durable rigid lids are made of other phenolic resins, and are available with a polytetrafluoroethylene (Teflon) liner, sold as PHEN-PTFE closures (see *Appendix III*).

Screw-on lids must have *continuous threads*. Screw-on lids with gaps in the threads do not provide an adequate seal to prevent the evaporation of fluid preservatives.

Thermoplastic polypropylene lids are flexible enough to provide a good seal, but durable enough to be long-lasting in collection use. I recommend using a polypropylene lid with a knurled edge and a Teflon® liner.

Why Do Rigid Screw-on Lids Come Loose?

The phenomenon of thermal expansion causes rigid lids to spontaneously loosen with fluctuations in temperature in collection storage. For example, the difference in thermal expansion between a glass jar and a metal lid is 10^{-5}/°C (1/10,000). A 2°C daily change in temperature in the collection storage room will cause a lid to back-off by half-a-turn in just three years (R. Waller, pers. comm.).

How to Improve Closure Seals

There are several ways that the seal on glass jars with threaded lids can be improved if you cannot afford to replace the closures:

1. Use a polyethylene liner or insert between the lid and the jar.
2. Wrap the jar and lid junction with polypropylene/acrylic (PPA) adhesive transparent tape (Steigerwald and LaFramboise 1994, 1996) (*Appendix III*).
3. Wrap the jar threads with Teflon® pipe joint tape.
4. Use a sheet of polyethylene film or Parafilm® between the container and the closure (Peden 1980).
5. Coat the jar threads with petroleum jelly (e.g., Vaseline®).

Plastic Containers

It is possible that clear polyethylene terephthalate (PET) containers may provide a low-cost alternative to glass containers (Walker et al. 1999). At present their variety and availability is limited (see *Appendix III*). These containers have not yet been adequately tested and evaluated for their use in collections. Acrylic (e.g., Plexiglass®) containers are unacceptable for long-term storage of specimens because they warp and crack, and because the preservative diffuses through the acrylic material (van Dam et al. 2000).

A comparison of plastic container materials is provided in Table 7.

Containers for Large Specimens

The traditional container for large specimens in herpetological collections was a large glass or ceramic crock (Zweifel 1966). These are no longer in production, but some are still in use in museums. They are difficult to seal properly, awkward to move, and are not an efficient use of space. In addition, the glaze on ceramic crocks eventually deteriorates from contact with the preservative, resulting in leakage (Smith 1965). Suzumoto (1992a) recommended using closed-cell foam weather strip tape or extruded polyethylene tape for replacement gaskets for large glass and ceramic containers.

Table 7. Plastic containers.

Name	Identifying marks	Characteristics
High density polyethylene	HDPE	Resistant to fixatives and preservatives; susceptible to UV embrittlement; good resistance to oxygen permeance
Low density polyethylene	LDPE	Unsuitable for collection storage
Polyethylene terephthalate	PET and PETE	Resistant to fixatives and preservatives, oxygen impermeable, lightweight
Polypropylene	P/P	Durable but flexible; very good for closures
Polystyrene	P/S	Rigid, clear. Permeable to water and oxygen. Good for storage of dry materials; susceptible to damage from compounds containing benzene rings
Polyvinyl chloride	PVC	Embrittles, offgasses acids, unsuitable for use in collections

The preferred container for large herpetological specimens is a stainless steel tank (see *Appendix III*). The major drawbacks to these tanks are that (1) the specimens cannot be seen without opening the tank, (2) tanks are hard to seal, (3) the compressible gaskets fail after a few years from contact with the preservative; (4) many specimens must be handled to find the one you want; and (5) stainless steel tanks are expensive. Tanks should be housed on platforms with casters for ease in moving them in the collection. Gaskets should be inspected regularly and replaced when they show signs of failure.

Tanks may be constructed from plywood and lined with epoxy or fiberglass (Dundee 1962), but these may not be as reliable as stainless steel tanks.

A lower cost alternative to tanks are plastic buckets (see Table 6). The most common currently in use are high-density polyethylene (HDPE) buckets or barrels. Legler (1981) first proposed the use of plastic containers in herpetology collections. Subsequently, spontaneous cracking of some of these containers was reported (Schueler and Aniskowicz 1982). Polyethylene becomes brittle when exposed to ultraviolet light. Containers that have been used outdoors for any length of time are not suitable for collection storage. Polyethylene containers should not be stored near windows, under fluorescent lights that lack ultraviolet (UV) filters, or near any other source of UV radiation. HDPE containers that have not been exposed to UV radiation and are protected from UV sources in storage are preservative resistant and will hold up well in collections. Purchase containers with good seals (e.g., threaded lids with gaskets). See *Appendix III* for sources of HDPE containers. As with any large container in the collection, the condition of the buckets and barrels should be monitored regularly.

Carts and Trolleys

To move containers of fluid preserved specimens safely, use a smooth-rolling cart or trolley. Carts and trolleys should (1) be sturdy enough to hold the weight of a load of containers of specimens without bending; (2) have a lip or other restraining device to keep containers from falling off; (3) be small enough to easily negotiate aisles and doorways in the collection storage area; and (4) have large wheels (preferably with pneumatic tires) for smooth rolling. Clark et al (1994) described a safety trolley for moving large containers of fluid preserved specimens.

ALLOCATION OF SPECIMENS TO CONTAINERS

A variety of standard sizes of jars and other containers should be available for use in the collection. Using containers of standard sizes makes the process of replacement of containers, closures, gaskets, and liners more cost efficient, and makes it possible to accurately plan collection expansion or relocation. Purchasing containers in large quantities directly from the manufacturer will reduce the cost per container.

The standard container sizes recommended for herpetological collections are listed in Table 8.

The smallest standard size container that will reasonably hold the specimen should be selected. The container must be large enough that digits and tails of the specimens inside it are not stressed, but not so large that excessive amounts of preservative and shelf space are used. Always maintain at least twice the volume of fluid as specimens (Zweifel 1966). Too many specimens in a container will result in (1) increased time to locate needed specimens; (2) unnecessary handling of specimens; (3) increased risk of damage to specimens; and (4) significant dilution of the preservative (Taylor 1981a).

The container label should be flat against the side of the container, without overlapping itself or any part of a specimen, so that it can be easily read from the outside.

Fill the container to a level between the shoulder and the lid, so that the specimens inside are well covered with preservative. An increase in temperature increases the air pressure in the headspace of a sealed jar, which results in stress on the closure. The internal jar pressure depends on the vapor pressure of the fluid and the thermal expansion rate of the jar, the fluid, and the air above the fluid (Horie 1994, van Dam et al. 2000). For example, at 20°C, ethyl alcohol has an expansion coefficient 40 times higher than glass, and water has an expansion coefficient 8 times that of glass. For water or ethyl alcohol, an increase in temperature means a rise in the fluid level in the jar, which causes the compression of the air in the headspace, which puts stress on the closure and seal. The ratio of fluid volume to headspace is important. One reason to fill containers is to reduce the amount of air available for oxidation, but the higher the jar is filled with fluid, the greater the compression of the air in the headspace, and the greater the internal pressure. Based on the work of Horie (1994), I recommend filling a container to 90% of its volume for ethanol-based preservatives, or to 95% of its volume for water-based preservatives to reduce stress on the closure from temperature fluctuations.

When all containers are filled to a uniform level, those that lose fluid from evaporation or leakage are detected readily. In the event that part of an exceptionally long specimen protrudes into the head space above the preservative, cover it with a layer of cheesecloth that has its edges below the level of the preservative, so that the specimen remains moist.

Some Tricks for Removing Jar Closures

Threaded Lids. When attempting to open a recalcitrant lid, grasp it so that your hand goes around the edge of the lid as completely as possible. This applies even pressure all around the edge. Wrap a wide rubber band around the lid to give a better gripping surface. If this fails, loosen the lid by inverting the jar and

Table 8. Recommended standard containers for herpetological collections.

Container type	Closure type	Sizes	Use of container
Glass jars	Polypropylene (P/P), continuous thread (CT)	8 ounce (0.5 pint) 16 ounce (pint) quart 1 and 2 liter 0.5,1,2,3 and 5 gallon	Fluid preserved specimens
Glass jars	Polypropylene (P/P), continuous thread (CT)	40 x 73 mm 50 x 85 mm 58 x 66 mm	Cleared and stained specimens in glycerin
Glass vials, screw cap, continuous thread (CT)	Polypropylene (P/P) or PTFE, continuous thread (CT)	14 x 45 mm 17 x 60 mm 21 x 70 mm 25 x 95 mm 28 x 70 mm	Fluid preserved, cleared and stained specimens
Glass shell vials	Polyester fiber plug (submerged in larger glass container with P/P closure)	15 x 45 mm 17 x 60 mm 19 x 65 mm 21 x 50 mm 21 x 70 mm 4 x 95 mm	Fluid preserved specimens
Stainless steel tanks	Compressible gasket, clamp lid	18 to 50 gallon	Fluid preserved specimens
Polyethylene buckets and barrels	Threaded lid with compressible gasket	5 to 50 gallon	Fluid preserved specimens
Polystyrene boxes	Polystyrene lids, unhinged or hinged	Variety, depending on skeletal material	Skeletons, dry skins
PET boxes	Hinged PET lids	Variety, depending on skeletal material	Skeletons, dry skins
Acid-free paper boxes	Acid-free paper	Variety, depending on skeletal material	Skeletons, dry skins
Polyethylene bags	Heat sealed	Variety, depending on skeletal material	Dry skins, very large skeletons

striking the lid squarely on a flat surface, or by tapping around the edge of the lid with a wooden stick.

Clamped Lids. Loosen the clamp, pull the bail off of the lid, and remove the lid. If the lid is stuck, twist it, tap around its edge, or carefully insert a knife blade between the gasket and the lid to break the seal. The use of bottle forceps for this purpose is common but not officially sanctioned.

General Instructions for Handling Fluid Preserved Specimens

Specimens preserved in fluid, if well cared for, will probably keep indefinitely. Improper handling, especially desiccation, can quickly undo the work of centuries of preservation and care.

When working with preserved specimens, frequently submerge them in a tray of the preservative (not water), or cover them with a cloth moistened with the preservative.

If a tail, limb or other appendage breaks off of a specimen, tie it to the venter with soft string (the type for stringing tags). Specimens with limbs broken but still attached should have the loose part tied to the body. Specimens with openings in the body wall large enough for body parts to come free should have the opening bound in string or sewn shut with a good quality thread.

The preservative surrounding the specimen is also part of the specimen. Change the preservative only when the solution presents a danger to the specimen (e.g., it becomes too acidic or too basic, or so discolored that it will stain the specimen). When changing old or contaminated preservative solutions, rinse the specimens in water, but do not soak them, before placing them in fresh alcohol.

When specimens are placed in containers, position them in the bottom of the container so that if the fluid level drops from evaporation or a leak, the specimens will not be damaged until the loss of preservative is severe. Of course, before it gets to the severe point, the alert collection manager will take steps to ameliorate the problem.

Specimens should not interfere with the placement of the label in the container. When returning specimens to a container, put them in one at a time, keeping the label properly aligned. Gently shake the specimens down with forceps if necessary, watching for spaces forming between specimens that might prevent some individuals from being nearer the bottom of the container than they could be.

General Instructions for Handling Larval Material and Eggs

With few exceptions, larvae and eggs are kept in a 10% formalin solution, buffered with two salts (see *Fixation and Preservation*). Work with formaldehyde only in a well-ventilated workspace, preferably in front of a fume collector. Wear eye protection and formaldehyde resistant gloves (see *Formaldehyde Safety*).

Keep specimens moist while working with them by frequently submerging them in a formalin solution. Larvae and amphibian eggs are exceptionally fragile. Handle them with a teaspoon rather than forceps (which will cut and tear the specimens).

Keep larvae in shell vials with their catalog tag visible from the outside. Fill the vial with preservative and plug it with polyester fiber, then submerge it in a jar of preservative. Larvae should be stored head down or lying flat to avoid damage to the tail.

Procedures for Handling Specimens in Glycerin

Glycerin preparations are usually cleared and stained and are very fragile. Keep cleared and stained specimens in individual containers. Use external labels on the vials.

Add a few crystals of thymol ($C_{10}H_{14}O$) to each vial of glycerin preserved specimens to prevent bacterial or fungal growth. Thymol is a toxic chemical. Use thymol only under a fume hod. Do not inhale thymol. Avoid skin contact with thymol dust or crystals. Wear eye protection when working with thymol.

ALLOCATION OF SPECIMENS IN THE COLLECTION

Specimens in standard sized containers (except types) with proper labels are shelved in the collection storage area when not being used. Specimens should be arranged alphabetically by family within each order. Within each family, specimens should be arranged alphabetically by genus and species. Within a species, specimens should be arranged by geographic locality. Specimens identified only to family are shelved after the last alphabetical unit in that family group. Although various "phylogenetic" arrangements are used in some collections, these are nonsensical, as a true phylogenetic arrangement would necessitate branching shelving. Use of an alphabetical system simplifies retrieval of specimens from the collection, thus enhancing collection use.

Each taxon on the shelf may be delimited with a spacer made of wood, metal, or plastic. These spacers should be about a half-inch square and the same depth as the shelf. Use of spacers makes the units in the collection more obvious, and reduces many common shelving errors. It is preferable not to split a taxon between two shelves unless the containers of the taxon occupy more than one shelf.

Specimen containers should be spaced on the shelves to allow room for collection growth without requiring massive rearrangement of the jars.

Containers of formaldehyde-based preservatives should be housed separately from other specimens.

Holotypes and Paratypes. Types should be kept in a separate, secure area of collection storage. Types are arranged alphabetically by genus and species without regard to family group. Type specimens are the most valuable material in the collection, and thus must be handled with special care. The specimen's type status is noted on the label by the word HOLOTYPE or PARATYPE. The scientific name on the holotype label is the original name for which type status is applicable, even if that name has been synonomized. Labels for paratypes may bear the current scientific name as long as the label is clearly marked PARATYPE of <Original name>.

Containers bearing holotypes are marked with a red ribbon tied around the neck of the jar or affixed to the outside of other types of containers. Containers bearing paratypes are marked with blue ribbon.

Specific permission must be obtained from the curator before types may be removed from their containers for examination or sent out on loan.

Large Containers. Large containers (stainless steel or wooden tanks, crocks, and buckets) should be numbered for identification. Keep a list of the catalog numbers of the specimens in each large container, organized sequentially. A small standard size jar with a dry label bearing the container number of the specimen should be housed with the regular collection to facilitate finding large container specimens. If possible, allocate specimens to large containers by some logical grouping (e.g., genus, family or order). Due to the expense, most collections have a limited number of large containers and their contents end up fairly well mixed after a few decades of specimen allocation. If specimens protrude above the level of the preservative, cover the parts with a layer of cheesecloth or soft double-washed cotton cloth to wick preservative over the specimen. Nylon mesh bags may be used to group specimens together within the large container. Do not overfill large containers with specimens—maintain the same 2:1 fluid volume to specimen volume that is maintained in small containers. Large containers are more susceptible to preservative loss from evaporation than are small containers, due to their increased surface to volume ratio and their seal length. Check the fluid volume of large containers regularly.

Specimens in Glycerin. Specimens in vials and small jars of glycerin are allocated to shelving, cabinets, or drawers arranged in the same order as the regular collection (Figure 11). Stand the vials upright in cardboard tubes (Ratcliffe and Messenger 1992) or in a block of ethafoam (Simmons 1992).

Dry Skeletal Material. It is preferable to mark the bones of skeletons with the catalog number. Use archival ink and an extra-fine tipped pen, and write the numbers on non-diagnostic parts of the bone. House skeletons in polystyrene or acid-free cardboard boxes with well-fitting lids. Arrange the boxes in trays or

Figure 11. Support system for vials.

drawers in cabinets in the same order as the regular collection. Small bones should be in small, lightweight vials made of polyethylene or polystyrene, or in gelatin capsules placed inside the boxes. Do not put glass vials in the containers with skeletons, as the movement of the vial will damage the bones. Stomach contents, skin, hyoid apparatus, and other fluid preparations from skeletonized specimens should be housed in vials in standard jars in the regular collection, not with the dry skeletons. Monitor skeletal specimens regularly for signs of pest activity.

Skeletal Material in Fluid Preservative. Identify the preservative in the specimen record. Small or fragile specimens should be placed in a shell vial with a polyester fiber plug, inside a standard size jar of preservative.

MUSEUM COLLECTION MANAGEMENT

It requires a very unusual mind to undertake the analysis of the obvious.—
Alfred North Whitehead (quoted in Curtis and Greenslet 1962)

The basic functions of collection management are:

1. To permanently associate the individual specimens with their data.
2. To maintain the specimens and data records in optimum condition, preserving the maximum amount of information obtainable.
3. To make the specimens and data available to qualified users.

While collection managers are ephemeral beings, collections and museums are comparatively eternal. The entries made in the catalog today, or the note scribbled on a piece of paper and stuck in a jar explaining what happened to the contents, must be interpreted after 50 or 100 or 200 years with the same veracity as the day they were written. Unfortunately, most of our mistakes become part of the collection, too.

Responsibility of the Collection Manager to the CollectionThe primary responsibility of the collection manager is the care and maintenance of the collection and collection data. Neglect will result in loss of or irreparable damage to specimens or data, or separation of the specimens from their data. Even the smallest, most casual errors may be perpetuated for years, and within a short time of their occurrence, many errors are impossible to correct. The collection manager must:

- Keep procedures logical and standardized.
- Protect the integrity of the collection and the data.
- Make specimens and data available for use.
- Keep records of all chemicals and procedures used to process and care for the specimens.
- Monitor the storage environment.
- Keep records of all collection activity.
- Strive to improve standards of collection management.
- Keep the collection and preparation laboratory organized and equipped.

Responsibility of the Collection Manager to the Collection UsersA systematic collection is an irreplaceable scientific resource. Properly documented specimens have intrinsic value, scientific value, and monetary value (Shelton and Simmons 2000). Collections are cared for by the institution, as a public trust (Malaro 1998).

It is presumptuous to think that we know all of the questions that can be answered by using preserved specimens and their data. Additional uses of properly preserved material will undoubtedly be found in the future. The collection manager must do all that is possible to keep collections in a state that will enable them to provide maximum usefulness in the future. Collection use requiring destructive or consumptive sampling must be carefully evaluated.

LOANS, GIFTS, AND EXCHANGES

Loan Policy

The institution should have a clear and concise policy regarding what will be loaned, to whom, for how long, and for what purposes (see *Collection Management Policy*).

Historically, most borrowers were workers in other systematic collections. Loaning institutions could assume that the borrower knew how to care for scientific specimens. Increasingly, many people are using systematic collections who may not know how to handle specimens and who may not have the facilities to care for them. A concise sheet of loan conditions and instructions for handling fluid preserved specimens should be included with each outgoing loan (see Figure 12).

GENERAL LOAN INSTRUCTIONS AND CONDITIONS

1. Please sign and return the marked copy of the loan invoice promptly.
2. The borrower agrees to protect and preserve material on loan.
3. Material must be returned by date stated on invoice unless a renewal request is granted.
4. Material on loan is the responsibility of the institution named on the invoice.
5. Material on loan may not be transferred or transported to another institution without prior permission.
6. Tags and labels may not be removed without prior permission.
7. Any dissections must be approved in writing.
8. Please acknowledge the loaning institution in any publications or reports based on borrowed material.
9. Please send a copy of any publication or report resulting from the use of this material to the loaning institution.

INSTRUCTIONS FOR HANDLING FLUID PRESERVED SPECIMENS

1. Maintain the specimens in the same preservative in which they are received (usually 70% ethyl alcohol or 10% buffered formalin).
2. Do not put specimens in denatured alcohol or any other type of alcohol without prior permission.
3. Do not submerge specimens in water.
4. Do not allow specimens to dehydrate.
5. Keep specimens away from heat.
6. Do not expose specimens to ultraviolet light.
7. Please pack specimens carefully for return shipment.
8. Please drain liquid from packages before sealing (do not ship specimens with a pourable amount of fluid in the package).

INSTRUCTIONS FOR HANDLING DRY SKELETAL SPECIMENS

1. Please take care to keep each skeleton together.
2. Do not clean bones without permission.
3. Please pad skeletal elements carefully for return shipment.

Figure 12. Loan Conditions and Specimen Handling Directions

Although there was a *quid pro quo* relationship with regard to collection resources, many collection users now offer little or nothing in return to the institution faced with the expense of maintaining the collection and making it available to the scientific community. Consider, for example, requests for tissue samples. The user rarely has collections to loan or exchange in return, and the use of tissues is destructive. In the future, museums will have to find ways to recover the costs of caring for specimens and making them available to those members of the community who do not contribute to the growth or maintenance of collections.

A *loan policy* should include the following concerns:

1. Specimens are loaned to institutions, not to individuals. Specimens on loan to an institution may not be transferred or removed from the institution to which they are on loan without written consent from the loaning institution.

2. Although loans are made to institutions, the individual borrower is still responsible for the welfare of the material on loan. Students wishing to borrow material must have a major professor co-sign their loan request.
3. Specimens on loan may only be used for the purposes for which they were loaned, unless written permission for a change is made.
4. Copies of loan invoices must be signed and returned promptly.
5. Written permission is required to make any modifications to a specimen, including opening the body cavity, removing anything from the body cavity, or preparing any parts. Any parts removed must be labeled and returned with the specimen.
6. Specimens must be properly packed for return to the loaning institution.
7. The entire loan should be returned at the same time, if possible. Large loans should be returned in more than one shipping container.
8. All taxonomic changes or re-identifications should be communicated to the loaning institution in writing. Copies of any publications or reports based on the borrowed material should be sent to the loaning institution.
9. Specimens should be cited in publications by catalog number, using the correct symbolic code (Leviton et al., 1985).
10. For a detailed discussion of conditions for outgoing loans, see Merritt (1992).

Categories of Loans

There are four categories of loan transactions:

1. Loans going to an outside borrower.
2. Receipt of loans returned by an outside borrower.
3. Receipt of specimens on loan for use by a member of the museum staff or visitor.
4. Return to sender of loans received for use by a member of the museum staff or visitor.

To this some museums add a fifth category—internal loans, for specimens used in the institution but outside of the administrative unit responsible for the collection.

Loan shipments entering or leaving the United States must be reported to the US Fish and Wildlife Service, using a 3-177 declaration form (see *Appendix I*). For shipments of non-CITES listed cataloged specimens from one museum to another, the 3-177 declaration must be filed with the appropriate Fish and Wildlife office within 180 days. Most institutions with heavy loan traffic file the necessary 3-177 forms twice each year; other institutions prefer to file the forms as each shipment is sent or received. If the shipment contains CITES listed species, the CITES permits must be obtained for the shipment and the 3-177 declaration filed at the time the loan is sent or received. The US Fish and Wildlife Service webpage has a listing of regional offices, contact information, 3-177 forms, and instructions available for downloading. The CITES webpage has a list of species covered under CITES and the CITES regulations. Both web pages are listed in *Appendix I*.

Loan transactions (for both the initiation and return of loans) should be numbered sequentially and invoiced for tracking and record keeping. The transaction invoice provides the legal documentation of the movement of the specimens. The museum copy of the invoice is archived as part of the museum's permanent records.

The following guidelines should be used when evaluating a loan request:
Number and Size of Specimens Requested. Is the number of specimens reasonable? Does it include all of the specimens in the collection of a particular taxon? Are similar specimens available in other institutions? What will the cost of retrieving, packing, and shipping the specimens be? Some specimens (e.g., frogs) are relatively easy to pack and inexpensive to ship compared to, for example, tortoises. Some large requests may be better divided into two or more separate loans, with one installment being sent upon the safe return of the previous installment. Most museums will not loan out all of their specimens of a single species at the same time.
Nature of the Specimens Requested. Are the requested specimens holotypes or paratypes? Are they specimens of species poorly represented in the collection, or an extinct species? Is the specimen a voucher for an audio recording or image?
Condition of the Specimens Requested. Are the specimens too old or fragile to be packed and shipped? Are they a preparation type that does not travel well?
Use of the Specimens. Is the receiving institution equipped to properly care for specimens on loan? What does the borrower intend to do with the specimens? Any dissections, molding and casting, photographs for publication, or use in exhibition must be approved in advance.
Project Design. Is the material requested really needed by the researcher? The project should be outlined in the letter of request. Is the borrower an over-achiever asking for an unreasonable number of specimens, or someone proposing an unrealistic project?
Loan Record of the Borrower. By keeping a borrower record of loan activity, you can evaluate the chances of seeing the loan returned within your lifetime. If a borrower already has a large amount of material on loan, request that it be returned before more is sent. If specimens have been mishandled or abused by the borrower, or packed improperly for return, you should educate the borrower or deny future loan requests.
Location of the Borrower. Particularly for large, hard to pack, or expensive to ship requests, it is sometimes preferable for the borrower to visit the museum rather than to send specimens on loan.
Priority Use of Material. Has another researcher reserved the specimens for a current, on-going, reasonable project?

The Loan Invoice

Loan invoices should be printed on standard size, white, acid free 100% cotton rag paper. The original (signed) loan invoices are part of the historical record of the activities of the museum, and should be archived. The loan invoice consists of four copies:

1. The first copy is maintained on file as a record of the transaction while the loan is active.
2. The second copy is mailed to the borrower separately from the specimens. The borrower checks the specimens against this invoice when the loan is received. This copy is signed and returned to the loaning museum, to be archived as a permanent record of the loan transaction.
3. The third copy is retained by the borrower.
4. The fourth copy is used as a packing list and is sent with the specimens.

Do not use self-carbonized paper or colored paper for loan invoices. These are not archivally stable. Self-carbonized forms fade remarkably quickly (Kortlucke 1981). Most institutions generate loan invoices using a printer connected to the database, and can thus produce multiple originals. The loan invoice should contain the following information:

1. Name, address, telephone and email address of the loaning institution.
2. Name, address, telephone and email address of the borrowing institution.
3. Name of person the loan is to be used by.
4. Date the loan was sent to the borrower.
5. Means of transport of the shipment to the borrower (e.g., US Mail, Federal Express, UPS, air freight, hand-carried).
6. Loan number.
7. Catalog number, scientific name, and locality data for each specimen in the loan.
8. Preparation type of each specimen and how it is to be housed (e.g., in 70% ethyl alcohol).
9. Date the loan is to be returned.
10. Space for borrower to sign upon receipt of the loan.

Procedures for the Preparation of Outgoing Loans

A good review of how to pack fluid-preserved specimens for shipment may be found in McCoy (1993). Specimens should be wrapped in a layer of cheesecloth (gauze) so that each specimen is covered with the material. The cheesecloth-shrouded specimens are then placed in a polyethylene bag, and moistened with the appropriate preservative. Each bag should be labeled and well sealed. No excess preservative should remain in the bag. If a bag leaks, the preservative can damage the shipping container. The bag of specimens is sealed inside a second plastic bag, then packed in a sturdy cardboard box for shipment, padded with packing material. Never pack a container so that a plastic bag of specimens is in contact with the outside wall of the box. Although specimens should be packed just prior to shipment and unpacked immediately upon receipt to avoid dehydration risks, well-packed specimens will survive several weeks in the bag.

1. *Select the specimens to be loaned.* Loan requests must be approved by the appropriate authority (usually the curator). Be sure that the person approving the loan is aware of any paratypes, holotypes, unique specimens, or exceptionally fragile specimens included in the request. Once approved, retrieve the specimens from the collection storage area and bring them to the preparation laboratory.
2. *Complete the loan form.* Have the complete copy of the loan form in front of you to check off the specimen's catalog numbers one-by-one as they are packed.
3. *Wrap each specimen* in at least one layer of cheesecloth. Several specimens may be rolled up in one length of cheesecloth. Keeping in mind the size of the plastic bags and packing containers available, spread out a layer of cheesecloth on a flat surface, then lay out the specimens so that fingers, toes, and tails will not be stressed (Figure 13).

Fold the edges of the cheesecloth over the specimens and roll the package up securely but not tightly. It may be necessary to pad sharp, protruding claws (e.g., turtles, some lizards) or spines (e.g., *Phrynosoma*; see Montanucci 1994). This can be done by wrapping the sharp parts with cheesecloth, or by padding them with polyester fiber and then wrapping with cheesecloth.

4. *Insert one or two rolls of wrapped specimens into a polyethylene bag.* Polyethylene for packing specimens can be ordered as individual bags or in long tubes to be sealed with a heat sealer (Loveridge 1952). If bags are to be

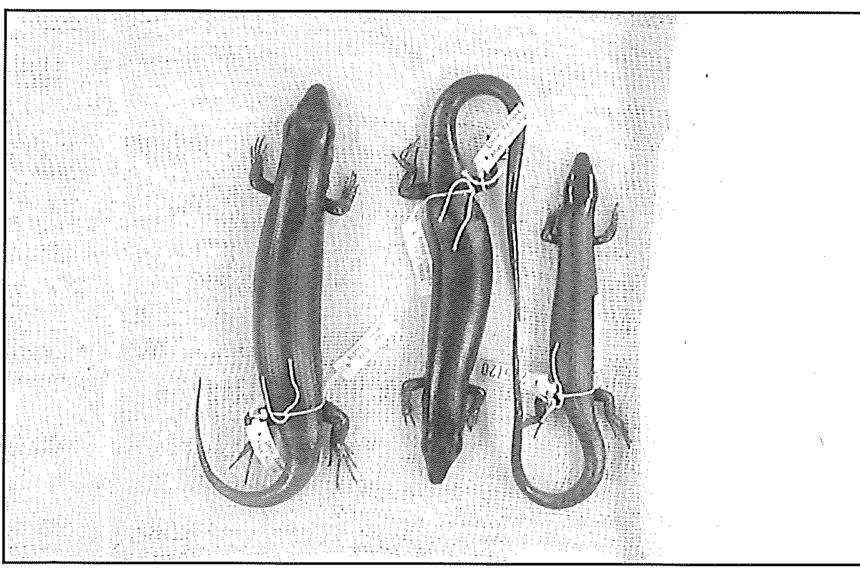


Figure 13. Postioning specimens for packing.

tied shut, use two mil polyethylene. If a heat sealer is used, select a four mil polyethylene. The thicker polyethylene provides better protection for the specimens. Do not attempt to close plastic bags using rubber bands (which deteriorate from contact with preservative) or with string (which stretches when wet). Do not use zipper bags (e.g., Zip-Loc®) or wire clamp bags (e.g., Whirl-Pac®), as these do not seal well enough for the stress of shipment. See *Appendix III* for suppliers of plastic bags, polyethylene tubing, and heat sealers.

5. *Pour a small amount of the appropriate preservative solution* into the bag with the specimens, and thoroughly moisten the cheesecloth. Carefully decant the fluid, so that no standing fluid remains in the bag.
6. *Seal the bag.* Use a heat sealer with a 1/8 to 1/4-inch wide seal width.
7. *Place the sealed bag inside another bag*, insert an address label between bags so that it is visible from the outside and seal the second bag (Figure 14). Depending on the thickness of the plastic and the size and weight of the specimens, a third bag may be appropriate as well.
8. *Larvae* should remain in fluid at all times to avoid dehydration. They should be packed in screw-top vials with good closures. An alternative is to use a loose packing of clean polyester fiber, moistened with the preservative, above, below, and around the larvae, taking care not to compress the specimen. Include a paper label bearing the specimen lot number inside the vial. After screwing the lid on tightly, tape the vial/lid junction with masking tape to prevent it from coming loose. Seal the vial inside two plastic bags.

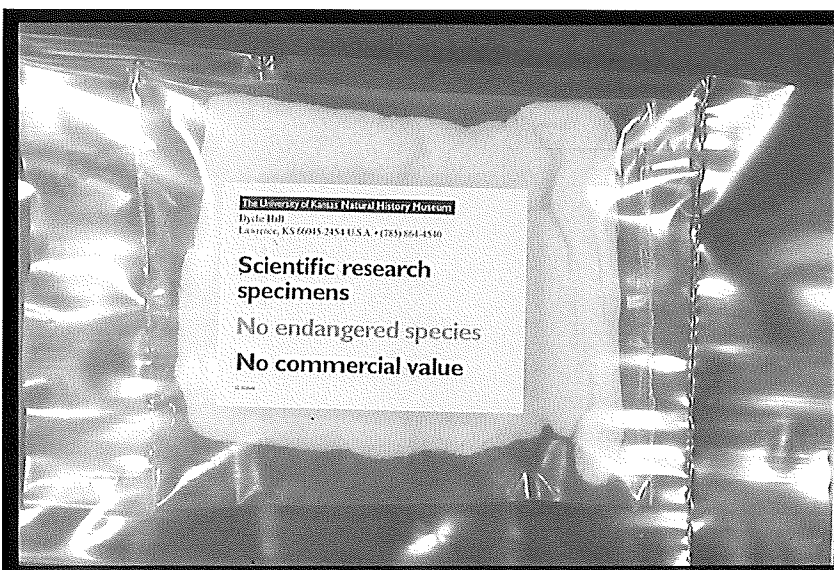


Figure 14. Packed specimens with address label.

9. *Dry skeletons* should be packed in a sturdy acid-free cardboard or polystyrene box with clean polyester fiber padding. Small bones should be placed in a gelatin capsule so they do not become lost in the packing material. Tape the lid to the box.
10. *Cleared and stained* specimens in glycerin should be shipped in a small vial of glycerin. Tape the lid to the vial and seal the vial inside two plastic bags.
11. *Pack the plastic bags of specimens in a sturdy cardboard box or can.* Do not allow any of the specimen packages to touch the outer wall of the container, as this would place them at greater risk if the container was damaged in shipment. Use a packing material that will maintain its bulk during shipment. I recommend the use of "plastic peanuts." Do not use the biodegradable packing pellets. These pellets are made of a cornstarch-based material that attracts pests and dissolves upon contact with preservative fluids or other moisture.
12. *Enclose a return address label* and shipping copy of the loan invoice inside the package.
13. If shipping CITES listed specimens, attach a copy of the institutional CITES permit to the package.
14. If shipping the package outside of the United States, complete customs declaration forms (2976 and/or 2976A) and attach them to the package. Describe the contents as "Preserved scientific specimens," and the value as "No commercial value." If a value is listed, the recipient may have to pay duty to retrieve the shipment from customs.
15. *Seal the container* with a sturdy, plastic box sealing tape. Do not use paper tape (which has humidity sensitive adhesives), masking tape (which has weak adhesives), or strapping tape (which rapidly deteriorates with age and exposure to UV).

Marking Containers of Specimens on Loan

Specimen containers and labels are never sent out with loaned specimens. The labeled container is marked to indicate that the specimen is on loan, and is promptly returned to the collection to serve as a placeholder for the borrowed material.

Containers from which some or all of the specimens have been removed for a loan should be marked with a small loan slip attached to the outside. The loan slip should contain at least the following information:

- The catalog number(s) of the specimen(s) borrowed.
- The loan number.
- The last name of the borrower.
- The date of the loan.
- The name or initials of the person who prepared the loan.

A sample format for a loan slip is shown in Figure 15.

SPECIMEN(S) WITHDRAWN FROM THE COLLECTION
Division of Herpetology, Natural History Museum

Numbers: ____

____

Loan Number: ___________________ Date: ____________________

Skin, skull, skeleton, Alcoholic, larvae

Withdrawn by: ________________________________

Figure 15. Container marker for specimens on loan.

Returning Loans to Senders

When specimens are returned to another institution, a return-of-loan invoice should be prepared. This sequentially numbered invoice is similar to the invoice used for outgoing loans. Include the original loan number on the document to aid the loaning institution in processing the return. The return of loan invoice should contain the following information:

1. Name, address, telephone number, and email address of the institution returning the loan.
2. Name, address, telephone number, and email address of the institution the loan is being returned to.
3. Date the loan is returned to the lender.
4. Means of transport of the shipment.
5. Return of Loan number.
6. Original loan number from loaning institution.
7. Catalog number and scientific name for each specimen being returned.
8. Space for a representative of the loaning institution to sign upon receipt of the returned specimens.

INTERNAL USE OF SPECIMENS

When specimens from the collection are used in-house by curators, staff, students, or visitors, they are usually removed from the collection for days to months at a time. Users should fill out an in-house specimen use slip and affix it to the container. If the entire container of specimens is removed to an office or laboratory, place the in-house use slip in an empty container of the same size, and shelve it in the collection to mark the spot where the specimens should reside. This procedure will aid in locating specimens that are being used and serve as a reminder to the collection manager to retrieve specimens from users in a timely fashion. The in-house use slip should be marked with the borrowed and returned dates and name of the borrower (see Figure 16 for a suggested format).

The completed in-house use slips are returned to the collection manager upon return of the specimens. These slips can be analyzed to obtain an index of collection use and to highlight areas of the collection that may need special evaluation of preservative concentration and container seals.

WITHDRAWAL FORM

Taxon: ______________________________

Number(s): ______________________________

____

Political Unit: ______________________________

Taken by: ______________________________

Temporary location of specimen: ______________

Date withdrawn: ______________________________

Returned by: ______________________________

Date Returned: ______________________________

Figure 16. Form for Internal Use of Specimens.

MIXING PRESERVATIVES

Preservative fluids should be mixed under a fume hood or in a well-ventilated area to prevent inhalation of the preservative vapors. Ground the container and the pump before dispensing bulk preservative (see *Fire Code and Safety Regulations*). Use distilled or deionized water to mix preservatives—tap water contains minerals and chemicals that will contaminate preservative solutions. Measure solutions in a clean, graduated container. Do not attempt to mix solutions by reading the giant meniscus in a 5-gallon carboy sitting on the counter. Let solutions to sit for at least 24 hr after mixing before they are used. During this time the solution will reach equilibrium and return to room temperature (solution temperature increases due to mixing the preservative with water). Check the strength of alcohol solutions, preferably with a digital density meter. A hydrometer may be used, but these are only accurate to within ± 5%. If neither of these is available, make an alcohol tester as described by Moore (1983, 1994). Refer to Waller and Strang (1996) for a chart showing density measurements, temperature, and volume percent of ethyl alcohol.

TOPPING UP CONTAINERS AND PRESERVATIVE CONCENTRATION

Because alcohol evaporates from solutions faster than water (Simmons 1995), as the fluid level drops in a container due to evaporation, the concentration of alcohol will also drop. Containers should be topped up with an appropriate concentration of preservative to achieve the desired storage strength. Continued topping up with stock solution will result in a lower than desired concentration of preservative.

Sendall and Hughes (1996) published a formula for use in correcting alcohol concentrations when topping up straight-sided containers. The formula is:

$$z(x + y) = ax + by$$

where *z* is the desired concentration of preservative (percent); *x* is the measured height of fluid in the container; *a* is the measured concentration of fluid (percent); *b* is the concentration of the stock solution being added (percent); and *y* is the amount of fluid to add (relative to container height). The equation is solved for *y*:

$$y = \frac{(z-a)x}{(b-z)}$$

For example, suppose you want a 70% solution of ethyl alcohol (*z*) in a container that has 11 cm of fluid in it (*x*). You are adding 95% ethyl alcohol while topping up (*b*), and you have determined that the present concentration in the container after fluid loss is 59% ethyl alcohol (*a*). Solving for *y* tells you that you need to add 4.84 cm of the 95% ethyl alcohol to bring the container up to the desired 70% concentration.

REHYDRATION OF SPECIMENS

After years of unhappy experiences, I have concluded that once a fluid-preserved specimen has become dehydrated, it should probably be maintained in that state unless it is absolutely necessary to rehydrate it in order to use it. Dehydration is very damaging to fluid-preserved specimens; rehydration will cause even more damage. Rehydration efforts are rarely successful, and will shorten the life of a specimen. In the event that it is absolutely necessary to rehydrate a dehydrated specimen, the techniques listed in Table 9 have been suggested. It is best to experiment on a junk specimen before attempting to rehydate a valuable specimen. Although various rates of success have been claimed, none of the published reports includes an analysis of the long-term preservation status of the treated specimens.

UPDATING OR MODIFYING SPECIMEN RECORDS

Post-cataloging specimen preparation, correction of locality or other collecting information, re-identification of specimens, synonomy of previous names, and the naming of new taxa are the principal reasons for needing to make changes to specimen records. When to make changes is often a judgement call for the curatorial staff. Generally, it is the responsibility of the curator, often in consultation with the collection manager, to make these decisions. Factors to consider include:

- Reliability of the person recommending the change.
- Nature of the new specimen preparation.
- Quality of criteria used in naming a new taxa or synonomizing an existing one.

Table 9. Rehydration techniques.

Technique	Reference	Comments
30% ammonia and warm water	Traditional technique	Poor results; ammonia damages proteinaceous tissues
2:1 solution of hydrogen peroxide and water	Traditional technique	Poor results; hydrogen peroxide damages tissues
3:1 isopropyl alcohol and water	Traditional technique	May initially soften specimen but results in further dehydration of the tissues over time
Surfactant (detergent) in water	Banks and Williams 1972	Generally poor results on badly dehydrated specimens; chemicals in the detergent will contaminate specimen for future chemical analysis
0.25 to 0.50% solution of commercial grade trisodium phosphate in water	van Cleave and Ross 1947	Usually unsatisfactory; trisodium phosphate is potentially damaging to tissues
0.5% trisodium phosphate and water for 24 hr	Marhue 1983	Usually unsatisfactory; trisodium phosphate is potentially damaging to tissues
Stage through solutions of 10%, 5% and 0.5% acetic acid, followed by trisodium phosphate solution	Vogt 1991	Arguably the most successful system, but acetic acid and trisodium phosphate will damage protein
50% propylene glycol or ethylene glycol solution	Marhue 1983	Unknown effects on specimen; glycols are unsatisfactory as preservatives
10% solution of enzymatic drain cleaner (e.g. Drano™, Liquid Plumber™) for 3 weeks	Vogt 1998	Very damaging to specimen tissues; never subject specimens to exposure to proprietary products of uncertain composition

- Likely acceptance of a synonomy or a new taxa by the scientific community.
- Publication date of the new name (it is unwise to use a manuscript name on a label or in a database, as the name may be circulated accidentally before it is published by the author).
- The opinion of the curator as an expert systematist.

Collection workers and visitors should fill out a specimen record change form (figure 17) and give it to the collection manager when a needed update or other modification in a specimen record is necessary. The collection manager then consults with the curator (if necessary) before making the change.

When making nomenclatural updates, change all specimen records. These records may include a database, container labels, and labeling of images or recordings. The nomenclatural change may be recorded in the permanent hand-written catalog by noting it in pencil.

Paratypes that have been synonomized may be identified on the label by the current name followed by the original name, as in the following example:

Gastrotheca riobambae
(Paratype of *Gastrotheca cavia*)

SPECIMENS EXCHANGED, GIVEN TO OTHER INSTITUTIONS, DESTROYED, OR LOST

The catalog and database should be annotated to record any specimen that is exchanged, given to another institution, destroyed, or lost. However, catalog numbers should never be re-assigned. Even when a specimen is destroyed, the catalog record of the specimen should be retained. This record is valuable information, even when the specimen voucher is no longer extant. When specimens are exchanged or given to another institution, record the institution's symbolic code (Levition et al. 1985) and the specimen's new catalog number in the hand-written catalog and database. When a specimen is destroyed or lost, briefly describe what happened so that when a researcher in 50 or 100 years comes looking for the specimen, its fate will be known.

GUIDELINES FOR COLLECTION GROWTH

> Peale even preserved an unusual fish without a head, deeming it worthwhile to preserve that much until more could be learned.
> —C.C. Sellers (1980)

The philosophy of collection growth was once quite simple—more was better. Any specimen from most anywhere was fair game for accession. If for no other reason, the rising cost of housing and information management for large collections have made this attitude obsolete, but ethical and legal considerations are also involved in collection growth. See Cato (1986), Fritts (1976), and Malaro (1998) for discussions of museum acquisition policies.

SPECIMEN DATA CHANGE FORM

Taxon: ____

Number(s): ____________________________________

Change identification to:

____

Change status to: _______________________________

Correct locality to:

____

Other: ____

NOTES

Name of researcher: _____________________________

Date: ___________________

Approved by: ___________________________________

Date: ____

Figure 17. Form for Changes to Specimen Data.

The following criteria should be carefully evaluated when presented with the opportunity to accession new material:

1. **Collection documentation**. Do the specimens come with adequate supporting documentation in the form of field notes, images, and recordings? Is the collector trustworthy?
2. **Specimen preservation**. Were the specimens adequately prepared in the field? Can you reasonably expect them to be useful scientific specimens in 50, 100, or 200 years in the future? Are the specimens worth the investment of time and materials necessary to process them and house them properly?
3. **Legal issues**. Does the donor provide appropriate legal documentation in the form of collecting, export, and import permits? If the specimens were collected outside the United States, does the documentation include a copy of the 3-177 declaration? The burden of proof that the specimens are legal lies with the institution receiving them (see Berger and Philips 1977, Berger 1980, Malaro 1998, and Tompkins 1998 for further discussion).

4. **Ethical considerations**. Were the specimens obtained in an ethical manner? Do they represent a last chance to preserve the biodiversity of a threatened ecosystem, or does their collection lend encouragement to the destruction of wildlife of a region? Is the addition of these specimens justifiable in terms of their potential scientific value?
5. **Need for the specimens**. Will this accession complement material already in the collection, or fill gaps in the collection? If the specimens would be of little use to the regular users of the collection, might the they be better deposited in another institution? Are there other institutions that might benefit more from receiving the specimens? Is it important to accept the specimens as voucher specimens (Lee et al. 1982)? Merritt and Lidgard (2000) proposed a risk management model for acquisition and accession.

Guidelines for Exchanges

There are two types of exchange agreements. A *one-time exchange* is worked out with specific numbers of specimens or specific specimens in mind. An *open exchange* is an ongoing process. Often museums will enter into open exchanges of paratypes in order to avoid having type material housed in only one collection, in case of disaster. The following considerations must be taken into account when entering into exchange agreements:

- The exchange is not completed until both parties have the material in question in their possession.
- The documentation for the exchange should spell out clearly any conditions, terms, or time limits on the exchange agreement.
- Documents relating to the exchange should be signed by representatives of both institutions and archived as part of the permanent collection documentation.
- Copies of field notes and other specimen data should accompany the specimen in an exchange.

EVALUATION OF COLLECTIONS

Good collection management depends on regular evaluation of the collection management program. Annual statistics should be compiled to evaluate collection growth and use, particularly to identify underdeveloped or underused parts of the collection.

Evaluation of the Collection Management Operation

Periodically (at least annually), the effectiveness of the collection management program should be evaluated (Simmons 1986b). The following aspects of the operation should be considered:

1. Does the preparation laboratory provide a safe and secure environment for working with specimens?
2. Does the collection storage area provide a safe, secure, and stable environment for the long-term care of the collection?
3. Does cataloging, processing, and allocation of specimens to the collection keep pace with incoming accessions?

4. Are specimens promptly returned to a safe and secure storage environment immediately after use?
5. Are new accessions appropriately documented?
6. Is collection documentation stored in an archival, retrievable manner?
7. What is the response time for loan requests? It should be no longer than two to three weeks.
8. What is the response time for requests for specimen data?
9. Are there requests for specimen data that your data retrieval system will not allow you to fulfill?
10. Has the growth of the collection since the last evaluation met expectations?
11. Do the collection care personnel stay busy? Do they have so much to do that the quality of the collection care provided is suffering?
12. Does the scientific community know that your collection exists? Evaluate user records to see if some parts of your collection are being overlooked. If so, take steps to correct this oversight.

Evaluation of collection management and the state of the collection can be accomplished using the Collection Health Index (CHI) (McGinley 1993, Williams et al. 1996). The CHI provides a way of rating the overall health of the collection by evaluation of storage units (in the case of fluid preserved collections, these might be shelves or shelf units) rather than individual specimen assessments. It provides a management tool that is useful for assessing, describing, and comparing collection needs across a wide variety of collections.

Price and Fitzgerald (1996) have proposed a system of five categories that reflect the value of the specimen based on scientific, cultural, and monetary considerations. This classification system can be used to direct resource allocation within a collection and to justify the collection to administrators.

Annual Reports of Collection Management Activities

At the end of each calendar or fiscal year, prepare a summary of the previous year's collection management activities. Even if this is not required, it is a useful way to analyze the collection management program and improve it. The information in an annual report should include:

- Number of accessions and total number of specimens accessioned.
- Number of specimens cataloged during the year.
- Number of outgoing loans and number of specimens loaned.
- Number of loans and specimens returned.
- Number of loans and specimens received for staff, student, or visitor use.
- Number of loans and specimens returned to other institutions.
- Number of visitors on collection business and total number of visitor days.
- Number of inquiries received from the public (e.g., snake identifications).

- Number of requests for non-collection herpetological information (e.g., requests for data from bibliographic resources).
- Number of requests for collection data and total number of records provided.
- Major collection management activities and improvements in the collection management operations.

To aid in tracking day-to-day collection management activities as well as compiling the information for reports, I recommend keeping a running list of activities by category on a clipboard in the preparation laboratory. Track the following categories:
Loans sent out (initiated). Include the loan number, name of the individual and borrowing institution, brief description of the loan contents, and initials of the individual who prepared the loan.
Loans returned (received). Include the loan number, the borrower's return-of-loan number (if any), date return was received, and initials of person unpacking and reinstalling the specimens.
Loans received for staff, student, or visitor use. Include the loaning institution's loan number, name of loaning institution, name of staff member, student, or visitor who requested the specimens, date of receipt, and initials of person who unpacked the loan.
Loans for staff, student, or visitor use returned to loaning institution. Include the return-of-loan number, the loaning institution's loan number, name of loaning institution, date of return of specimens, and the initials of person who packed the specimens for return.
Receipt of gifts and exchanges. Include the date of receipt, name of sender, sender's institutional affiliation, brief description of specimens in shipment, and initials of person unpacking the shipment.
Requests for collection data. Include the date that the requested data was sent, the method of data transfer (e.g., email, hard copy), general description of data (e.g., hylid frogs from South Carolina), number of specimen records included, and initials of person responding to the data request.
Requests for non-collection information. Include the date the requested information was sent, method of information transfer (e.g., email, hard copy), general description of information (e.g., photocopy of a type description), and initials of person responding to the information request.
Inquiries from the public, tours, etc. Include the date, number of members of the public served, and initials of person dealing with the inquiry or tour.
Visitors. Include the date(s) of visit, purpose of visit (for visitors on collection-related business), and institutional affiliation of visitor.

The nature of a collection management program is progressive and dynamic. Frequent evaluation will help assure that your program is able to respond to the changing demands from the scientific community, while still protecting the collection for future use.

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APPENDIX I. SOURCES OF INFORMATION FOR COLLECTING AND IMPORT PERMITS

A. Where to Obtain Information on What Permits Are Needed

United States Fish and Wildlife Service Resources

Home page of the United States Fish and Wildlife Service
http://fws.gov

Fish and Wildlife Service Permits page
http://permits.fws.gov

National Park Service Research and Collection Permits
http://science.nature.nps.gov/research

US State Permits

Levell, J.P. 1997. *A Field Guide to Reptiles and the Law.* Second edition. Serpent's Tale Natural History Books, Lanesboro, Minnesota. 270 pp.

Permit-L discussion and information group

To sign up, send a message to LISTSERV@SIVM.SI.EDU with the message subscribe PERMIT-L <Your Name>.

B. Designated Ports Requirement (United States Fish and Wildlife Service)

US Fish and Wildlife Service Designated Ports (50 CRF 14.12)

Atlanta, Georgia
Baltimore, Maryland
Boston, Massachusetts
Chicago, Illinois
Dallas/Fort Worth, Texas
Honolulu, Hawaii
Los Angeles, California
Miami, Florida
New Orleans, Louisiana
New York (JFK), New York
New York/Newark, New Jersey
Portland, Oregon
San Francisco, California
Seattle, Washington

Designated Port Exemption Permits

Applications for Designated Port Exemption Permits must be made in advance of the importation or exportation through your Regional US Fish and Wildlife office. The addresses for the regional offices may be found on the US Fish and Wildlife web site at http://fws.gov.

C. *Useful References Related to Permits*

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Tompkins, W.G. 1998. Fish and Wildlife. Pp. 321-339 *in* Buck, R. A. and J.A. Gilmore (editors). *The New Museum Registration Methods.* American Association of Museums, Washington, D.C. xvii + 427 pp.

D. Convention on International Trade in Endangered Species (CITES)

CITES Home page
http://www.wcmc.org.uk/CITES/english/index.html

CITES listed species
http://www.wcmc.org.uk/CITES/english/fauna.htm

APPENDIX II. FIELD WORK LISTS

This appendix contains a selection from the on-line publication, ***Field Work Lists: Everything You Will Need for Camp or Trail*** by John E. Simmons and Mark Robbins. The complete publication is available at: http://nhm.ku.edu/birds/collections.html (click on "Preparing for Field Work").

INTRODUCTION

The only thing worse than arriving at camp and discovering that you do not have something you need, is returning home and finding that you have been lugging around something that you didn't need. To help avoid these problems, we have compiled the following lists of items. These lists are to be used as checklists when you are preparing for a field trip. The lists are based on our combined years of experience in fieldwork, and those of our friends.

Not everyone needs all of these things, of course. These lists are intended to be guidelines for planning. Whenever possible, check with a recent traveler to be sure that what you need can be purchased at your destination. If there are items critical to your trip that you are unsure of, take them with you.

HINTS FOR HAPPY PACKING

- Never waste space; small bundles enable you to use space more efficiently than do large bundles.
- Pack small things inside larger things.
- Remove most packaging from packaged goods.
- When padding around a liquid nitrogen tank, use only things which can be frozen and thawed safely (e.g., use towels, not a plastic drop cloth).
- Keep a detailed list of everything you pack in each bag or box (this will both help you locate items easily and file an accurate missing baggage claim).
- Take extra baggage labels with you.
- Use your work address and telephone number on all baggage, never use your home address.
- Be sure to obey airline rules for weight and dimension restrictions on luggage, especially for carry-on luggage.

MONEY AND HOW TO SPEND IT

Traveler's cheques are generally the safest way to transport money. Select a major brand. Check with a travel agent before heading for the field. Occasionally it will be impossible to change certain brands of traveler's cheques in some countries or smaller cities. Never try to take a cashier's check, international postal money order, or anything else other than traveler's cheques or cash. You will spend hours of valuable time trying to exchange them for local currency, and most often will not be able to.

Always carry your funds in a money belt or money pouch securely on your person. Do NOT leave cash, traveler's cheques, or credit cards in a hotel room, in a camera bag, or in a secret compartment of your suitcase. NEVER carry money, credit cards, or anything else of value in a wallet in a hip pocket, in a purse, or in a fanny pack. Use a money belt or pouch for all money and credit cards. Do not carry all of your funds in the same place on your person. Spread funds among the members of your party, or use more than one money belt or pouch.

Empty your wallet of everything you will not need (e.g., library card, extra credit cards) before you depart for the field.

BEFORE YOU GO

Ask your colleagues for personal contacts in the country you are headed for, and then contact those people when you arrive. You may need their help later. Go to the public library and copy the entries from a large encyclopedia (recommended: *Encyclopaedia Britannica*) and from a good guidebook for the country you are headed for, and read these on the plane. It is always useful to have a bit of historical and cultural perspective on the place you will be working so you can converse knowledgeably with the citizens you deal with.

WHAT SHOULD YOU CARRY IN YOUR CARRY ON?

Passport, identification that shows your institutional affiliation (e.g., institutional identification, letter from the museum director on letterhead, business cards), toothbrush, toothpaste, aspirin, change of socks and underwear (in case your baggage does not arrive with you), luggage keys, money and traveler's cheques, camera and film. Consider also carrying with you anything small but of high value (e.g., GPS unit, binoculars, prescription medications). Carry along a bottle of water to drink when in-flight service is not available, reading matter, and of course, your airline tickets.

CAMP GEAR

bottle opener
candle holders
candles
can opener
chair (folding)
coffee pot (for boiling water)
cook stove, portable (recommended: propane)
cooking utensils
cup (for drinking; recommended: cup with lid)
detergent (dish washing)
dinnerware (plates, glasses, mugs, forks, spoons)
dishcloth
eating utensils (forks, spoons)
entrenching tool (folding shovel)
file to sharpen machete
filter system for drinking and cooking water (Level II system with microbiological micron rating of 0.2 to 1.0)
flagging, plastic (safety orange, for marking trails)
ground cloth for tent
hammer or ax for driving in stakes
hammock
knife

lantern (recommended: propane)
lantern mantles
lighter, disposable
machete
machete scabbard
matches
measuring spoons
mosquito netting
nylon cord (to secure tents and tarps, hang bags)
plastic bags, very large (e.g., Hefty™ trash bags)
plastic bags, small (e.g., Ziploc™)
polyethylene sheeting (to cover gear while traveling, etc.)
potato peeler
propane tank for lantern
propane tank for stove
rope
saucepan (two quart) and lid
scouring sponge
skillet
small broom
spatula
table, folding
tape, box sealing
tape, duct
tape, electrical
tape, masking
tarp, nylon
tent
tent stakes (two extra)
toilet tissue (0.3 roll per person per day)
water jugs (two; one for water ready to drink, one for water being treated)

*Even if sleeping indoors, you should either sleep under mosquito netting or inside a tent, especially if indoors is under a thatch roof.

PERSONAL ITEMS

airline tickets
alarm clock (battery operated)
baby powder (to dry feet)
backpack
basin plug, universal (for plugging hotel sinks)
belt
belt, utility
blouse (conservative, for wearing when you apply for permits; select a material that dries quickly)
books for recreational reading (recommended: inexpensive editions with larger type for reading in dim light)
boot inserts
boots, hiking (break in before the trip)
boots, rubber (with good support; break in before trip)
calculator (small)
canteen

clothes line
clothes pins
coat (recommended: water repellent; avoid military-style clothing)
comb
contact lens case
contact lens cleaning supplies
cot
cotton sheets
credit cards
day pack (note: do NOT wear a day pack in the city)
deck of cards
dental floss
deodorant
dress clothes (conservative, for wearing when you apply for permits)
ear plugs (both to protect hearing on noisy city streets and to help you sleep)
electrical converter (if traveling in areas without 110 volt current)
emory board (nail file)
eyeglasses
eyeglass repair kit
extra pair of eyeglasses
face mask (for sleeping in light rooms)
facial tissues
fanny pack (note: do NOT wear fanny backs on your butt in the city; wear them in front only)
flashlight
flashlight batteries
flip-flops (shower shoes)
frisbee
hair brush
hand cream
handkerchief or bandana
hand wipes (moist towelettes, anti-bacterial; for cleaning up before eating or while traveling)
hat for rain
hat for sun
insect repellent [diethylmetatoluamide (DEET) in liquid form or as pump spray]
jacket, light weight (avoid military-style clothing)
keys, extra, to anything you have which locks (have another member of your party carry your extra keys)
knife, hunting
knife, folding (e.g., Swiss Army™ knife. Don't skimp on this—buy the one with the most gadgets)
laces (extra) for boots and shoes
laundry bag
mirror, small (recommended: stainless steel)
money belt or money pouch (neck or calf type)
nail brush (useful for both fingernails and for clothing)
nail clippers
neck pillow (inflatable)
pants (of material that dries quickly, e.g., cotton or khaki)
passport (make sure that it is still valid)

photo ID (e.g., valid driver's license)
photographs of family and home (these are great to start conversations with foreigners you meet)
pillow
pillow case (stuff with extra clothing to make a pillow)
poncho, water repellent
pajamas
Q-tips
radio, AM/FM
radio, portable short-wave (recommended: Gundig™ or Sony™)
raincoat
razor
razor blades
safety pins (assorted sizes)
sanitary napkins
sewing kit (with extra buttons)
shampoo
shoes
shorts
shirts, long sleeve (of material that dries quickly; avoid military-style clothing)
shirts, short sleeve (of material that dries quickly; avoid military-style clothing)
sink plug (frequently missing in lower priced hotels)
skirt (conservative, for wearing when you apply for permits)
sleeping bag
sleeping pad
soap
soap dish
shoes, city
shoes, walking/hiking
socks, cotton
socks, woolen (for wear on cold nights)
sunglasses
sweater
swimsuit
tampons
toothpicks
T-shirts
toothbrush
toothbrush case
toothpaste
towels
umbrella
underwear
Vaseline™
Walkman™ type cassette player and selection of entertaining music
washcloth
watch
windbreaker

TOOLS*

multi-tool (e.g., Original Leatherman™, Gerber Multi-Plier™ or well-made copy)
oil
hose clamps (adjustable, variety of sizes)
pliers, regular
pliers, needle-nosed (with wire cutter blade)
screwdriver set, slot and Philips (recommended: one handle with multiple blades)
vice grips (small)
WD-40 (small container)
selection of a few small screws, bolts, nuts, and washers to repair suitcases, travel boxes, and other items

*Consider the equipment you are taking. Bring along the appropriate tools (e.g. Allen wrenches) to do whatever repairs you will be capable of.

MEDICINE CHEST*

adhesive tape (surgical tape)
acetaminophen
aloe vera cream for minor rashes, irritations, burns and scrapes
antacid tablets (e.g., Rolaides™, Tums™)
antibacterial cream, 3-in-1 type (recommended: Mycitracin™)
antibiotics (e.g., ampicillin, tetracycline)
antidiarrheal [recommended: Lomotil™ tablets (diphenoxylate; only by prescription), Imodium™ (loperamide), Pepto Bismol™ (bismuth subsalicylate)]
antihistamine (with chlorpheniramine maleate)
anti-malarial drugs (these can be hard on the liver, make certain you need them, consult with local health center; usually recommended are Chloroquine™ and/or Fansidar™)
aspirin
baking soda (make paste to relieve itching, minor burns; 1/2 tsp in 8 oz of water is a good antacid)
Band-Aids™ (sticking plasters)
bandage tape
Betadine™
charcoal (for stomach gas)
Chiggerex™
cloth sling
decongestant (pseudoephedrine, e.g., Sudafed™)
dental floss
Dramamine™ (dimenhydrinate, for motion sickness)
ear drops
Elastoplast™ strip
ethyl alcohol (95%, for sterilizing)
emory board (can also be used for filing broken teeth)
eye wash
flea powder
foot powder, anti-fungal [e.g., Desenex™ or Tinaderm™(tolnaftate)]
gauze compresses, sterile
gauze pads, sterile
ginger root capsules, 400 to 500 mg (for stomach gas)

hepatitis vaccination
lip balm
menstral pads (for use as compresses)
moleskin
oral analgesic [benzocaine (ethyl p-aminobenzoate) based; e.g., Oragel™]
skin cream (for rashes and insect bites; recommended: benzocaine and aloe vera)
snake bite kit (recommended: Extractor™ from Sawyer)
sulfur powder (mix 50:50 with baby powder for chiggers)
sun block
suture thread (sterile)
tension bandage
thermometer
vitamins (esp. vitamin C tablets, B-12 complex)
tetanus shot (renewed every 5-10 years)
Tiger Balm™ (external analgesic with methyl salicylate and menthol)
topical antiseptic
zinc oxide ointment (for sunburn or skin rash; e.g., Desitin™)

*You will find that in many countries you can buy antibiotics and prescription strength pain killers over-the-counter.

*Carry your medications in a proper looking bottle (e.g., a prescription medicine bottle) so it will not look like contraband.

*If you do get diarrhea, be careful not to become dehydrated. The following formula is recommended by the World Heath Organization: to one liter of clean water, add 3.5 g sodium chloride, 1.5 g potassium chloride, 20.0 g glucose, and 2.9 g trisodium citrate. A simpler formula if you cannot get those ingredients: to one liter of clean water, add 1/2 tsp salt, 4 tbs sugar. Follow up by eating yogurt (once the diarrhea stops) to restore intestinal fauna.

*Immunizations before your trip: recommended hepatitis, polio, typhoid, tetanus, and yellow fever.

GENERAL FIELD AND COLLECTING GEAR

address book with phone and FAX numbers of museum and several colleagues
altimeter
binoculars
camp chair, folding
camera battery, extra
compass
cryo pen, extra fine point (Sharpie™; for marking cryo tubes)
cryo tubes
cryo tube holder
dictionary (of appropriate foreign language)
dissecting kit
dissecting needle (for marking cryo tubes)
envelopes, large (for receipts, permits, etc.)
eraser (for pencil)
field book (recommended: 3-ring binder)
field book paper (acid free)

field box (recommended: high impact molded plastic, with locks)
file (for sharpening machete)
film (both slow and high speed, color and black & white; recommended: Kodachrome 64, Fuji Velvia 200, Ektachrome 400, T-Max 100 and 400)
flash battery, extra
flash for camera
flashlight, large (6-volt; recommended: Mag-lite™)
flashlight, small (AA size; recommended: Mini Mag-lite™ style)
global positioning device (GPS unit) (with extra battery)
ink (archival) for technical pen (recommended: Higgins™ T-100 Drafting Film Ink)
institutional envelopes and address labels
institutional letterhead
keys, extra, for all locks
logbook for receipts
maps
marker, permanent (recommended: fine point and blunt point Sharpie™ markers)
measuring tape
mesh bags, nylon (for food and other supplies in camp)
notebook, pocket size (for carrying on the trail and in the city)
padlocks for baggage (including field box)
pen, disposable [recommended: Marsgraphic Pigment Liner™ (Staedtler); Uniball Deluxe MicroPen™ (Faber Castell); Permaroller™ (Pentel); avoid acidic inks]
pen, technical
pencil, no. 2 lead
pencil sharpener
pencil, mechanical
pencil leads for mechanical pencil (recommended: HB)
photo scale (for placement in photos)
plastic tape in various colors
sharpening stone (for pocket knife)
sketchbook
sleeping bag (select weight and fill carefully, based on destination)
stool (folding)
stopwatch
thermometer, maximum/minimum
thermometer, pocket case
thermos for liquid nitrogen
psychrometer
rain gauge
references for field identifications

TISSUE SAMPLING GEAR

aluminum foil
aquarium net
buffer solution to back up frozen samples
catalog (for tissue samples)
cryo marker
cryo tubes
cryo tube holder
cryo tube marking pen (Sharpie™, extra fine point)

ethanol (95%, in squeeze bottle, to clean dissecting gear between samples)
ethanol, absolute
ice chest
forceps (keep clean for use with tissues)
Kimwipes™
liquid nitrogen tank
liquid nitrogen (check availability before leaving on trip)
nylon stockings
paper towels
plastic tubes, large (heavy duty, to fill with water and freeze in nitrogen tank for return trip)
scalpel (keep clean for use with tissues)
scalpel blades (keep clean for use with tissues)
scissors (keep clean for use with tissues)
strainer
string
tongs

HERPETOLOGY GEAR

benzocaine (ethyl p-aminobenzoate) based oral analgesic (e.g., Oragel™)
blowgun
blowgun darts
bottle forceps
buffer for formalin (pre-measured capsules of dibasic and monobasic salts)
calipers
cheesecloth
Chloretone™ (hydrous chlorobutanol)
chloroform
cloth bags (variety of sizes)
dip net
ethyl acetate
ethyl alcohol
fishhooks
fishing pole, collapsible (for noosing lizards)
forceps, bottle
forceps, placental
forceps, small
formalin-resistant gloves
formaldehyde
gallon plastic jug with wide mouth
gloves, work (recommended: leather palms and fingers, canvas back)
head lamp (with beam that can be focused; recommended: Justrite Electric Head Lantern™)
head lamp bulbs (extras)
Lidocaine
microphone (recommended: Sennheiser™)
microphone batteries
monofilament
MS-222 (tricaine methesulfonate)
Nembutal™ (aqueous sodium pentobarbital)
paper towels

plastic bags, large
plastic bags, medium
plastic bags, small
pocket magnifier (6x-10x)
pocket microscope (30x)
preserving trays, plastic, with snap-on tops
repair kit for formalin resistant gloves
rubber bands, very large
ruler (small)
scales, electronic (portable)
scales, spring
scalpel blades
scalpel handle
scissors, bandage
scissors, small
sewing needle (suitable for tagging snakes)
slingshot
slingshot ammunition
snake hook
snake tongs
stump ripper
syringes and needles (do NOT pack these in your personal gear!)
tags, blank
tags, field series (pre-numbered)
tag string
tapes, cassette
tape recorder, cassette
tape recorder batteries
thread
tricaine methesulfonate
turtle traps
vials (screw cap; large selection of sizes)
work tray

APPENDIX III. SOURCES OF SUPPLIES AND INFORMATION

A. FIELD GEAR

General Collecting Gear

Ben Meadows (wide variety of equipment including portable electronic scales)
3589 Broad Street
Atlanta, GA 30341
800-241-6401
www.benmeadows.com

BioQuip
17803 La Salle Avenue
Gardena, CA 90248-3602
310-324-0620
www.bioquip.com

Cabela's Hunting, Fishing and Outdoor Gear
400 E Avenue A
Oskosh, NE 69190
800-237-444
www.cabelas.com

Forestry Suppliers (wide variety of equipment including portable electronic scales)
PO Box 8397
Jackson, MS 39284-8397
800-647-5368
www.forestry-suppliers.com

Miltex Surgical Instruments (placenta forceps with serrated ring jaws)
6 Ohio Drive
Lake Success, NY 10042
800-645-8000

Nichols Net and Twine Co., Inc.
2200 Highway 111
Granite City, IL 62040
800-878-6387

Herpetological Collecting Gear

Blowguns Northwest
PO Box 847
Lake Stevens, WA 98258
425-377-1496
888-774-0124
www.blowgunsnw.com

Bush Herpetological Supply
PO Box 539
Neodesha, KS 66757
800-451-6178

Furmont Reptile Equipment
Fuhrman Diversified
905H South 8th Street
La Porte, TX 77571
713-474-4832

LLL Reptile and Supply Company, Inc. (24" hooks and tongs)
609 Mission Avenue
Oceanside, CA 92054
760-439-8492
www.LLLReptile.com

Valentine, Inc.
4259 S. Western Boulevard
Chicago, IL 60609
800-438-7883

Plastic Bags, HDPE Buckets

Consolidated Plastics, Inc
1864 Enterprise Parkway
Twinsburg, OH 44087
800-362-1000

National Bag
2233 Old Mill Road
Hudson, Ohio 44236
800-247-6000

Preserving Trays

I recommend good quality polypropylene trays, such as Rubbermaid® brand. Quality polypropylene trays will be marked with the letters "PP," and are available at houseware and hardware stores, as well as from plastic container companies.

Snakebite Kit

TheExtractor®
Sawyer Products
PO Box 7036
Long Beach, CA 90807
213-423-0405
(widely available at sporting goods stores and from the general field gear suppliers listed above)

B. COLLECTION MANAGEMENT SUPPLIES

Fixative and Preservative Supplies

Alcohol, Formaldehyde and other chemicals

Contact chemical suppliers in your area.

Alcohol Testing Equipment (digital density meters, hygrometers)

Cole-Parmer Instrument Company
625 East Bunker Court
Vernon Hills, IL 60061-1844
800-323-4340
www.coleparmer.com

Fisher Scientific
P.O. Box 14989
St. Louis, Missouri 63178-4989
800-325-4075

Ethyl Alcohol Permits

Bureau of Alcohol, Tobacco, and Firearms
National Revenue Center
550 Main Street
Cincinnati, Ohio
800-398-2282

Safety Supplies and Spill Kit Supplies

Fisher Scientific
P.O. Box 14989
St. Louis, Missouri 63178-4989
800-325-4075

Lab Safety Supply
PO Box 1368
Janesville, WI 53547-1368
800-356-0783
Safety TechLine 1-800-356-2501
labsafety.com

Test Kits (e.g., EM Science Formaldehyde Test Kit)
See *Lab Safety Supply*

Conservation Supplies (UV filters, ethafoam, acid-free paper, acid-free envelopes for photographs and documents, mylar enclosures to archive tags and labels, dataloggers and hygrothermographs, etc.)

Light Impressions
PO Box 787
Brea, CA 92822-0787
800-828-6216
www.lightimpressionsdirect.com

Gaylord Brothers
PO Box 4901
Syracruse, NY 13221-4901
800-448-6160
www.gaylord.com

University Products
517 Main Street, P.O. Box 101
Holyoke, MA 01041-0101
800-628-1912
www.universityproducts.com

Containers

HDPE Buckets

See *Plastic Bags, Plastic Tube Bags, Heat Sealers, HDPE Buckets*

Gaskets *(for glass-top clamp jars)*

Manufactured Rubber Products
4501 Tacony Street
Philadelphis, Pennsylvania 19124
215-533-3600

Potomic Rubber Company, Inc.
9011 Hampton Overlook
Captial Heights, Maryland 20743
301-336-7400

Jars and Vials

Kols Containers, Inc.
1408 De Soto Road
Baltimore, MD 21230
800-457-5657

Chelsea Bottle Company, Inc.
10 Wesley Street
Chelsea, MA 02150
617-884-2323

Carolina Biological Supply
2700 York Road
Burlington, North Carolina 27215
800-334-551

Fisher Scientific
P.O. Box 14989
St. Louis, Missouri 63178-4989
800-325-4075

Qorpac (phenol resin and PHEN-PTFE closures)
www.qorpak.com
(available through major distributors such as Fisher Scientific)

Jar Closures (screw-top, flexible polypropylene)

Kols Containers, Inc.
1408 De Soto Road
Baltimore, MD 21230
800-457-5657

Chelsea Bottle Company, Inc.
10 Wesley Street
Chelsea, MA 02150
617-884-2323

Jar Sealing Tape

Polypropylene/acrylic-adhesive transparent tape
3M Product #5086

Steel Tanks

Delta Designs
PO Box 1733
Topeka, KS 66601
785-234-2244

Steel Fixtures
612 E 7th Street
Topeka, KS 66601
785-233-8911

Viking Metal Cabinet Company Inc.
5321 West 65th Street
Chicago, IL 60638
Contact: Linda Gottfried
800.776.7767
www.vikingmetal.com

Plastic Bags, Plastic Tube Bags, Heat Sealers, HDPE Buckets

Consolidated Plastics, Inc
1864 Enterprise Parkway
Twinsburg, OH 44087
800-362-1000

National Bag
2233 Old Mill Road
Hudson, Ohio 44236
800-247-6000

Specimen Tags

National Tag Company (Paxar)
815 S Brown School Road
Vandalia, OH 45377
800-543-7580

Allen Bailey Tag and Label Company
One Main Street
Whitinsville, MA 01588
800-724-1069

Storage Supplies

Paper Tubes (for vial storage)

Jonesville Paper Tube Corporation
4740 Beck Road
P.O. Box 39
Jonesville, Michigan 49250
517-849-9963

Skeleton Boxes

Rigid polystyrene clear plastic boxes

Durphy Packaging Company
47 Richard Road
Ivyland, PA 18974-1512
215-674-1260
www.durphypkg.com/

Althor Products
496 Danbury Road
Wilton, CN 06897
800-688-2693
www.thomasregister.com/olc/althor/main.htm

Cardboard boxes, acid free

All Packaging
1515 W 9th Street
Kansas City, Missouri 64191
Phone 816-842-3711
FAX 816-842-8312

Thermal Printers

Alpha Systems, Inc.
13509 E. Boundary Road
Midlothian, VA 23112
800-849-9870
http://www.preservationtag.com

C. SOURCES OF COLLECTION MANAGEMENT INFORMATION

American Society of Ichthyologists and Herpetologists (*Curation Newsletter*, *Guidelines for Field Use of Amphibians and Reptiles*)
http://199.245.200.110/

Biology Curator's Group (UK)
www.bcg.man.ac.uk

National Park Service (*Conserve O Grams* and *Museum Handbook*)
http://www.cr.nps.gov/museum/publications/index.htm

Museum Documentation Association (*Fact Sheets* and other resources)
http://www.mda.org.uk/

Society for the Preservation of Natural History Collections (*Collection Forum and SPNHC Newsletter*)
http://www.spnhc.org

D. INSTITUTIONONAL ANIMAL CARE AND USE COMMITTEE GUIDEBOOK

http://grants.nih.gov/grants/olaw/GuideBook.pdf

E. ON-LINE TAXONOMIC SOURCES

Amphibian Species of the World
http://research.amnh.org/herpetology/amphibia/index.html

EMBL Reptile Database
http://www.embl-heidelberg.de/~uetz/LivingReptiles.html

NOTES

Herpetological Circulars

(Currently Available)

1. *A Guide to Preservation Techniques for Amphibians and Reptiles* by George R. Pisani. Pp. 1-22. July 1973.
4. *A Brief Outline of Suggested Treatments for Diseases of Captive Reptiles* by James B. Murphy. Pp. 1-13. August 1975.
6. *Longevity of Reptiles and Amphibians in North American Collections (First Edition)* by J. Kevin Bowler. Pp. 1-32. May 1977.
7. *Standard Common and Current Scientific Names for North American Amphibians and Reptiles (First Edition)* by Joseph T. Collins, James E. Huheey, James L. Knight and Hobart M. Smith. Pp. 1-36. May 1978.
8. *A Brief History of Herpetology in North America Before 1900* by Kraig Adler. Pp. 1-40. May 1979.
9. *A Review of Marking Techniques for Amphibians and Reptiles* by John W. Ferner. Pp. 1-42. 1979. .
10. *Vernacular Names of South American Turtles* by Russell A. Mittermeier, Federico Medem and Anders G. J. Rhodin. Pp. 1-44. July 1980.
11. *Recent Instances of Albinism in North American Amphibians and Reptiles* by Stanley Dyrkacz. Pp. 1-32. May 1981.
12. *Standard Common and Current Scientific Names for North American Amphibians and Reptiles (Second Edition)* by Joseph T. Collins, Roger Conant, James E. Huheey, James L. Knight, Eric M. Rundquist and Hobart M. Smith. Pp. 1-28. June 1982..
13. *Silver Anniversary Membership Directory for the Society for the Study of Amphibians and Reptiles*. Pp. 1-55. July 1983.
14. *Checklist of the Turtles of the World with English Common Names* by John Iverson. Pp. 1-14. July 1985.
15. *Cannibalism in Reptiles: A Worldwide Review* by Joseph C. Mitchell. Pp. 1-37. October 1986.
17. *An Annotated List and Guide to the Amphibians and Reptiles of Monteverde, Costa Rica* by Marc P. Hayes, J. Alan Pounds, and Walter W. Timmerman Pp. 1-67. [1989].
18. *Type Catalogues of Herpetological Collections: An Annotated List of Lists* by Charles R. Crumly. Pp. 1-50. [February] 1990.
19. *Standard Common and Current Scientific Names for North American Amphibians and Reptiles (Third Edition)* by Joseph T. Collins. Pp. 1-41. July 1990.
20. *Age Determination in Turtles* by George R. Zug. Pp. 1-28. August 1991.
21. *Longevity of Reptiles and Amphibians in North American Collections (Second Edition)* by Andrew T. Snider and J. Kevin Bowler. Pp 1-40. June 1992.
22. *Biology, Status, and Management of the Timber Rattlesnake (Crotalus horridus): A Guide for Conservation* by William S. Brown. Pp. 1-78. February 1993.
23. *Scientific and Common Names for the Amphibians and Reptiles of Mexico in Spanish and English* by Ernest A. Liner. Pp. 1-113. June 1994.
24. *Citations for the Original Descriptions of North American Amphibians and Reptiles* by Ellin Beltz. Pp. 1-44. December 1995.
25. *Standard Common and Current Scientific Names of North American Amphibians and Reptiles (Fourth Edition)* by Joseph T. Collins. Pp. 1-40. April 1997.
26. *Venomous Snakes: A Safety Guide for Reptile Keepers* by William Altimari. Pp. 1-24. September 1998.
27. *Lineages and Histories of Zoo Herpetologists in the United States* by Winston Card and James B. Murphy. Pp. 1-45. February 2000
28. *State and Provincial Amphibian and Reptile Publications for the United States and Canada* by John J. Moriarty and Aaron M. Bauer. Pp. 1-52. July 2000
29. *Scientific and Standard English Names of Amphibians and Reptiles of North America North of Mexico, with Comments Regarding Confidence in Our Understanding.* Committee on Standard English and Scientific Names. Pp. 1-82. November 2000
30. *Amphibian Monitoring in Latin America: A Protocol Manual/Monitoreo de Anfibios en América Latina: Manual de Protocolos* by Karen R. Lips, Jamie Reaser, Bruce E. Young and Roberto Ibáñez. Pp1-115. Septmeber 2001

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