FAUNA *of* AUSTRALIA

14. COLLECTION AND PRESERVATION OF THE REPTILIA

Terry D. Schwaner & Chris B. Banks

One of the most important aspects to consider before undertaking any collection is to obtain the requisite permits from the relevant authorities. In terms of the Constitution, control of the collection of Australian fauna is the reponsibility of the six States and two mainland Territories which comprise The Commonwealth of Australia. There are a number of administering authorities in each State or Territory and it is essential to obtain the permission of each relevant authority before collecting begins. Permission to enter any land is a separate issue which collectors should clarify with landowners before collecting. This applies especially to Aboriginal lands and heritage areas.

To assist researchers, a detailed guide to requirements for collecting Australian plants and animals is available (ABRS 1993). The guide provides the contact personnel and addresses of State and Commonwealth authorities responsible for issuing collection permits and licenses. Copies of this guide may be obtained from the Australian Biological Resources Study, GPO Box 787, CANBERRA ACT 2601; telephone (06) 250 9440 or 250 9443, facsimile (06) 250 9448.

Import and export of legally collected material from Australia requires strict observance of international conventions, such as the Convention on International Trade in Endangered Species (CITES). Further information may be obtained from the (****check title and address****) Chief Executive Officer, Australian Nature Conservation Agency, GPO Box 636, CANBERRA ACT 2601; telephone (06) 250 0200 within Australia, or +61 6 250 0200 from overseas.

Collectors of reptiles in Australia should coordinate their collecting activities with curators of the State collections in their respective areas. These collections are now computerised to provide a quick response to requests for species identifications and distributions. Most State museums now have modern facilities and equipment that may be loaned to assist others in their collecting and preservation efforts.

The frustrating and potentially dangerous nature of collecting reptiles emphasises a need for clear objectives and proper planning when taking reptiles from the wild. Knowledge of the animals' habitats, climatic preferences, and lifestyles will enhance success. Crocodiles and turtles tend to be diurnal, active animals, although the former are most often encountered at night. The saltwater crocodile (*Crocodylus porosus*) is the most aggressive Australian species and great care is needed when dealing with individuals in excess of 3 m in length. Subtropical and tropical reptiles are active year round, whilst species from more temperate southern areas become dormant (or at least restricted in activity) over the colder winter months.

Slowly driving or walking through potential collecting areas is advisable, especially when snakes and lizards are likely to be active. Diurnal species are usually active in the early morning (0630 to 1000 hours) or late afternoon (1600 to 1800 hours) in warmer seasons. However, many lizards will be active throughout the day, and activity times will vary considerably seasonally (for example, Schwaner 1989), geographically, or between local habitats (for example, with light or dark coloured soils; Heatwole 1976). Nocturnal species, particularly some lizards and snakes, are especially active on warm nights following rains.

The exact location, date and collector of each specimen are as important as the specimens themselves. No collector should attempt to take reptiles without being able to locate the animals' capture sites on a map. Where established roads or trails are lacking, distances (in km) from various topographic features, grid coordinates or latitude and longitude must be recorded. Readings from the recently developed Global Positioning System are ideal. Features of the

environment and the behaviour of each specimen should also be recorded. Simmons (1987) and Garrett (1989) discuss the types of information required for collected specimens.

Housing and feeding of reptiles in captivity are beyond the scope of this chapter. However, an overview of the topic is given by Cogger (1992; see also references therein).

CAPTURE

Hand capture

Freshwater turtles can be collected occasionally by hand from shallow, clear, still or slowly moving water. When disturbed, they usually dive to the bottom or hide under overhanging banks and aquatic vegetation. Some turtles habitually sit on the bottom, directly below where they entered the water, and, if the water is especially deep or dirty, they can be captured by skin diving or with SCUBA equipment (Cann 1978). Freshwater turtles are less apt to flee if approached indirectly under water. Marine turtles are best caught when they come ashore to lay eggs at night. They can be captured at sea also, by jumping onto them while they rest at the surface, a technique termed ‘rodeoing’.

Freshwater and saltwater crocodiles, *C. johnstoni*, and *C. porosus*, up to 1.2 m in length, can be caught by hand from a boat or an airboat (Walsh 1987). However, for larger individuals, collecting equipment should be used to avoid serious injury to all concerned.

All lizards can be captured by hand, but large monitor lizards can inflict painful bites and geckos are subject to damage by rough handling. Hand collecting during cooler hours of the day will be more productive for diurnal lizards with preferred high body temperatures, particularly if the species hides among stones or rock slabs. Small specimens can be coaxed out of rock crevices or tree hollows by gentle prodding with a length of wire. A crowbar can be useful for lifting large rocks (Swanson 1976).

Importantly, habitat disturbance must be kept to a minimum when searching for lizards and snakes. The combination of many physical, chemical and biological processes results in the micro-community which supports the reptile or its prey. Replace logs, rocks and even man-made debris in their original positions, and avoid damage to living or dead vegetation.

Trenches associated with construction sites and shallow, abandoned wells, are usually worth checking for lizards and snakes that may have fallen in overnight. Inexperienced collectors are warned, however, that venomous snakes may present a particular hazard in such confined situations.

Non-venomous file snakes, *Acrochordus arafurae*, may be caught by feeling amongst submerged vegetation (Shine & Lambeck 1986), and pythons should be grasped quickly behind the head or gently around the body (for less aggressive individuals). However, venomous snakes should not be caught by hand. Though some elapids can be picked up by the tail and deposited in bags or bins, the technique is not recommended, even for experienced snake handlers. A snake stick, also known as a pinning stick, jigger or Head Pinning Device (Ehmann 1975) provides the safest method of catching venomous snakes. Essentially, this is a 1 to 2 m length of metal tubing (about 20 mm in diameter) with a wide fork, or a wooden stick (25 mm in diameter) with a T-shaped section, at one end. A leather strap stretched tightly between the forks, or a piece of foam rubber glued to the T-shaped section, allows the snake’s head or neck to be firmly pinned from above, without injury (Fig. 14.1A).

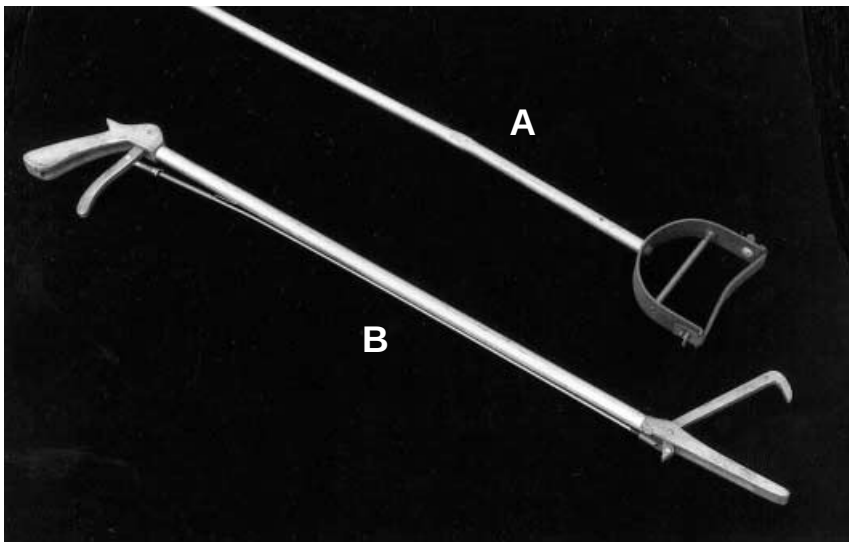

Figure 14.1 Implements used to capture snakes. **A**, head pinning device, showing the leather strap stretched across the fork of the base; **B**, a grab-stick, which can be closed on the snake remotely, by squeezing the handle. [Photo by C. Glasby]

Other methods, including a simple L-shaped jigger, may be used to pin, hook and lift the snake, and, if strong enough, can also be used to pry under bark or small stones. Grab-sticks can be useful for large, swift, venomous snakes. This implement is a 1 to 1.2 m length of aluminium tubing with padded jaws at one end and a hand operated lever mechanism on the other end to close the jaws quickly on the moving snake.

Once the head or neck is secured, the snake is then quickly, but carefully, grasped behind the pinning stick with the thumb and first two fingers of one hand. The snake is gently pulled until the thumb and fingers reach and feel the angle of the jaw bones (or quadrate area). Once the snake is held firmly, the stick can be removed and the snake lifted from the ground or surface. As many snakes will remain quiet until the pinner is removed and then attempt to jerk free, it is best to grasp the snake's body with a free hand when lifting it, while maintaining the hold on the head and a cautious watch on the snake. A second person should be available to assist at all times when handling and examining a venomous snake (Fig. 14.1B).

Two methods are recommended for placing a venomous snake in a bag by hand. The first should be considered for snakes that are small relative to the bag size. An assistant holds the bag open and above the ground while the snake handler lowers the U-shaped body into the bag, using both hands to hold the head and tail. On an agreed signal, the handler quickly drops the snake into the bag and, simultaneously, grabs the top of the bag from the assistant (with either free hand), swirling the bag vigorously, both to drive the snake to the bottom and to twist the top of the bag down on the snake. Lowering the bag to the ground prevents it from unwinding and a stick placed over (at right angles to) the twisted portion can be held in place with a foot while the bag is tied. The hoop bag method (Gow 1989) is safer because the bag is large (1.2 to 1.5 m deep) and the assistant initiates twisting. The snake's body and then the head are lowered into the bag and the head is then carefully grasped with a free hand from outside the bag. The initial hold is released, that hand withdrawn from the bag, and the bag secured with ties before the second hold is released (quickly).

Snakes are best carried in strong calico or linen bags that have been double-seamed along the bottom and sides, and across the bottom corners. Two tying tapes should be sewn below the bag's opening and bags with specimens should always be handled above the tied area—snakes can inflict a bite through the bag. Other bag designs incorporate zippers for quick removal, or use sheer material so that the snake's position can be seen at all times (Weigel pers. comm.). When transporting snakes in bags, consider the need to protect them from injury, or extreme heat and cold.

Trapping

Simple wire drum nets, supported by a strong wire frame or internal cross-supports and with a capacity of about 200 litres, work well for freshwater turtles. A funnel-shaped entry hole, facing inwards, allows the turtle entry but prevents its escape. Bait may be tied to an internal wire or placed in a smaller internal cage. The trap can be tied to the bank, suspended on poles in the water, or from an overhanging branch, and the top 50 to 100 mm of the trap must protrude above the water to allow the trapped animals to breathe. A smaller version which operates on the same principle is shown in Figure 14.2. Other techniques include a plank fixed across the mouth of a large floating barrel, in which a few holes have been bored (Pritchard 1967), or a floating platform encircling a central wire-netting cage (Cann 1978), are alternative designs.

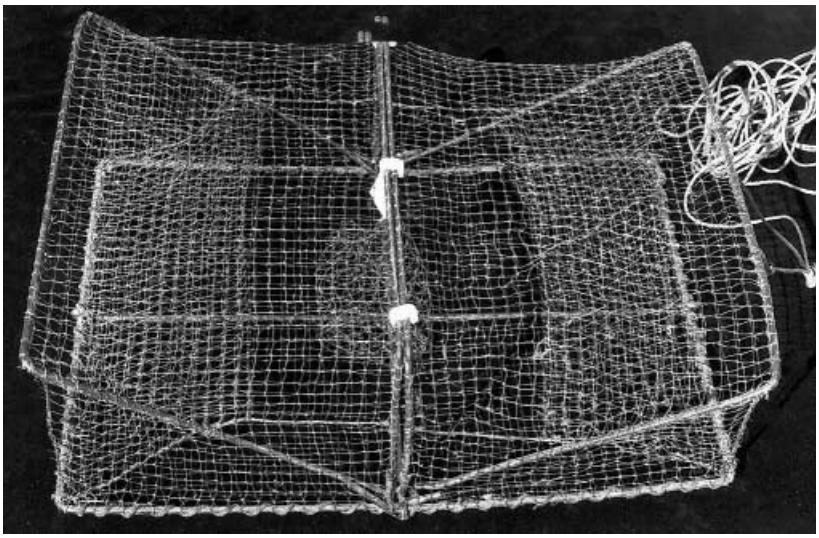

Figure 14.2 A wire netting trap for freshwater turtles. A horizontal, wedge-shaped entry funnel leads inwards from each end; the bait pocket is visible beneath the central bars. [Photo by C. Glasby]

Traps are used for first-time collecting of large crocodiles in habitats that prevent the use of other methods, and for mark-release-recapture studies of growth and movement. An easily constructed, portable, cylindrical trap of steel mesh sections was designed by the Conservation Commission of the Northern Territory (Walsh 1987) to capture crocodiles up to 4.5 m total length. Attachable floats allow its use in tidal areas.

Rope traps can be used for crocodiles longer than 5 m in length, to avoid injury in steel traps. These traps take considerable time to construct and require a heavy counterweight, such as a large tree, close to the mouth of the trap when the bait is pulled. The trap can be left alone until it is sprung if a radio alarm, currently available with a range of 30 to 40 km, is used (Webb & Messell 1977).

Pit-traps are effective for collecting small to medium-sized lizards, wandering freshwater turtles and some small snakes (Braithwaite 1983; Mather 1979). A variety of plastic and metal buckets, ice cream containers, steel tubs and PVC piping have been used. The lip of the trap should be flush with the ground surface and great care should be taken to ensure that the surfaces leading to the trap edges are similar to those of the surrounding habitat. Metal sheets, bark strips or large flat rocks, elevated by small stones or sticks to allow animals to crawl under them and into the trap, will also protect trapped animals from sunlight. The efficiency of pit-traps is greatly increased when a drift fence is used (Morton, Gillam, Jones & Fleming 1988) (Fig. 14.3).

Live mammal traps, such as metal Sherman traps will catch small diurnal and nocturnal, terrestrial skinks, and the larger 'bandicoot' traps are effective for large monitor lizards (Cogger 1992). Shine (1977a) used wire funnel traps successfully to catch elapids in eastern Australia—a method often used in other countries for terrestrial snakes (for example, Seigel 1986; Fitch 1987). Similarly, an aerial 'drift fence', consisting of a 15 m ö 1 m diameter tube of fine wire mesh, with funnels at either end, and supported 3 to 15 m above ground in tropical forest, has been successful for catching small Central American iguanids and colubrids (Vogt 1987).

All traps should be monitored at least daily. When not monitored, traps should be covered with tight-fitting lids to prevent the needless death of a wandering animal. If lids are not available, pieces of board, sticks or rocks can be placed in the trap to allow a trapped animal to escape. In well-drained areas, small holes in the bottom of pit-traps will prevent water accumulation and subsequent drowning of trapped individuals.

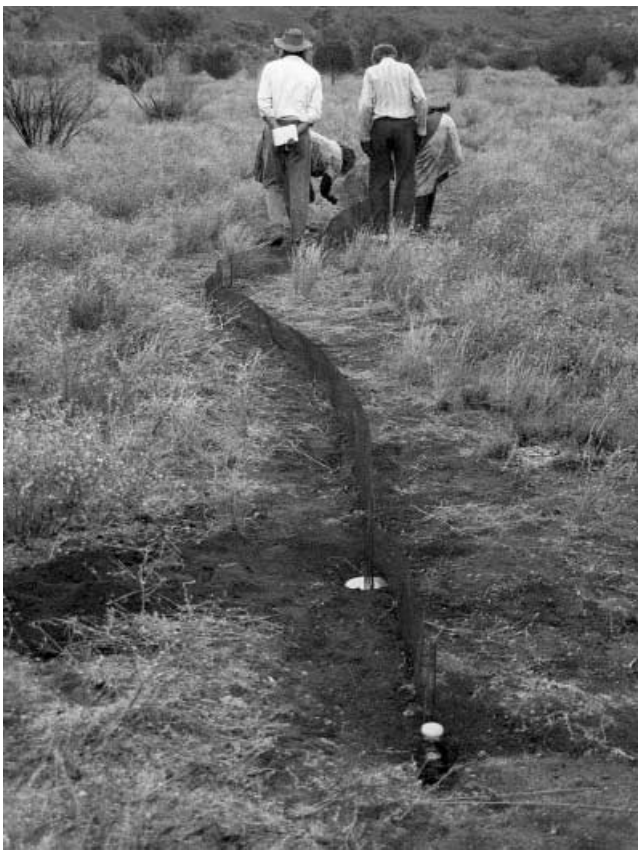

Figure 14.3 Routine check of pit-traps, in use with a drift fence. The white container buried in the foreground will trap animals, which fall in after wandering along the fence. [Photo by Lesley Muirhead]

Harpooning

Webb & Messell (1977) used a small harpoon head on a sturdy pole attached to about 50 m of line to capture *Crocodylus porosus*. Crocodiles are approached at night with a spotlight and the harpoon head is jabbed into the soft skin of the neck or the tail of small or wary specimens. Skill is required to make a small wound only, and to avoid the impenetrable osteoderms on the crocodile's back (Walsh 1987).

Netting

Floating nets with a 60 to 80 mm stretched mesh size and cord diameter less than 1 mm have proved successful for catching *C. johnstoni* (Webb, Manolis & Buckworth 1983). Such nets must be checked regularly. Heavy duty commercial fishing nets have also been used to catch *C. porosus*, but are not often effective (Webb & Messell 1977). Freshwater turtles can be caught with gill nets, drag-nets and seine nets with reasonable success, but care must be taken to avoid drowning the turtles, and these methods have potentially disruptive effects on aquatic vegetation and other species. Freshwater turtles can swim under these nets if they are not set close to the bottom, or the animals may bury in the mud to escape capture.

Noosing

Quick-moving dragon and monitor lizards can be caught effectively using a long, light bamboo, aluminium or fibreglass pole, with a slip noose attached securely to one end (Swanson 1976; Madsen & Loman 1987). Portable models have interlocking segments for quick assembly and disassembly in the field.

Snares have been used successfully for *Alligator mississippiensis* and for wary *Crocodylus acutus* (Mazzotti & Brandt 1988; Webb pers. comm.). These techniques have not been applied to Australian species.

Another device, the noose-tube, consists of an aluminium tube (for strength) inside a hollow plastic tube (0.46 to 0.60 m long and 50 mm diameter), and a length of nylon cord affixed and looped at one end, with the other extending through and to the opposite end of the tube. The loop is placed over the head of a lizard or snake and the free end pulled to pin the animal's neck against the end of the pole. Grab sticks and noose-tubes are available commercially and widely used for capturing venomous pit-vipers in the United States (King & Duvall 1984), but home-made versions have been used successfully in Australia (Hutchinson pers. comm.).

Other Live-capture Methods

Stout rubber bands when stretched and fired from between the thumb of one hand and the thumb and forefinger of the other, can stun (or kill) small reptiles (especially lizards). This method is very effective at a distance of 3 to 4 m (or closer), and usually requires that the animal be positioned against a hard surface for maximal impact of the band. Skill is required to prevent killing the animal or breaking its tail. Eye protection should be worn to prevent injury as a result of backfire.

Tinkle & Lawrence (1956) successfully used a 1.2 to 1.5 m length of 16 mm bore aluminium tubing and a close fitting cork (painted with nail polish to increase its visibility and retrieval) as a blow pipe to stun small teiids and iguanids. More lethal versions use a carpet needle inserted into the narrow end of the cork. A water pistol, loaded with water, can be used to knock geckos from walls and rock-faces (Branch 1988).

Though apparently popular in other countries, jaw snares, Pitman snares and stockades (Hutton, Loveridge & Blake 1987) have had minimal success for catching crocodiles in Australia.

Baited hooks can be used to catch turtles, but this can injure animals and the hooks should not be left unattended. A slower and more humane method is to use a line and baited handnet, without a hook, to slowly drag the turtle into grasping range.

Kill-capture

Methods designed to kill-capture reptiles are not recommended unless a small sample is required. Animals killed in this way quickly deteriorate and must be processed to preserve viable tissues, gut contents, internal organs, whole specimens or even skeletal materials. A more effective method for collecting lizards and snakes is to shoot them with .22 dust shot (or 'rat shot'), fired from a pistol or rifle. Cogger (1992) preferred a pistol for animals at close distances to prevent excessive mutilation, and recommended the use of solid .22 bullets for large lizards or arboreal snakes. Small, mouse- or rat-traps, baited with large insects, have been used to catch iguanid lizards in the tropics of Central and South America (Heatwole, Maldonado & Ojasti 1961).

PRESERVATION & STORAGE

Traditional methods of preservation include wet specimen preparation and skeletal collections. These are essential for long term preservation and storage of type specimens, and for vouchers and samples that document geographic ranges, variation in anatomical characters (for example, colour, pattern, scutellation, internal organs, teeth and bones), reproductive condition, and habits (for example, prey in stomach contents). Several excellent references (for example, Hall 1962; Pisani 1973; Simmons 1987, Cogger 1992; and references therein) provide guidelines and detailed directions for traditional preparation of reptile specimens.

Non-traditional methods are used to preserve viable tissues for cellular, chromosomal and molecular studies. These methods allow a greater range of potential characters for systematic analysis, and thereby extend the usefulness of classically preserved materials. Viable tissue collections, first established in Australia in 1980 by the Herpetological Section of the South Australian Museum (Schwaner 1982), are now present in the Australian Museum, Sydney, and the Western Australian Museum, Perth. Richardson, Baverstock & Adams (1986) provide more details on the collection and preservation of viable tissues and a comprehensive overview of molecular systematic methods is given by Hillis & Moritz (1990).

Although some morphologists regard tissue collecting as only necessary for an immediate project and some molecular systematists ignore the value of morphological features, we favour a balanced approach in which each specimen is carefully processed for tissues, without destroying internal and external anatomy (Schwaner 1982). What is compromised in this effort is time, simply because it takes longer to process a specimen for tissues, in addition to traditional methods of preservation.

Cooper, Ewbank, Platt & Warwick (1989) have addressed the critical issue (more generally considered by Wake, Zweifel, Dessauer, Nace, Pianka *et al*. 1975) of how to kill captured reptiles prior to preservation. Lethal injection with aqueous sodium pentobarbital, called 'Nembutal' or 'Lethobarb', is universally preferred, because death is painless, and is also accompanied by relaxation of the muscles, which facilitates fixing specimens in prescribed positions. However, this narcotic is highly regulated and generally restricted for public

use. Similarly, anaesthetic inhalants (for example, chloroform, succinylcholine chloride, trichloroethylene, and ether) require special handling and care as all are volatile and highly flammable. Drowning small specimens in warm water or freezing them are less satisfactory, because they take longer and involve a degree of discomfort to the animal. However, these methods are effective, less painful than pithing, and do not damage the specimen. The proper use of firearms to dispatch large reptiles is quick and effective (see Cooper *et al*. 1989), but this method obviously causes damage to specimens.

Wet Preparation

Dead reptiles (kill-captured or otherwise) deteriorate rapidly in even moderate ambient temperatures. Although the onset of putrification can be slowed by chilling, our experience is that all but the freshest road-killed specimens will soon become infested with maggots. The onset of *rigor mortis*, the appearance of a deep green patch on the belly (indicating a ruptured gall bladder) and a tell-tale odour are good signs that a dead individual has begun to rot. To retard this process, the tissues must be perfused with a preservative that kills bacteria, inactivates digestive enzymes and hardens soft parts.

A 10% solution of formalin—one part of a 40% saturated, or concentrated, solution of formaldehyde gas in water, and nine parts water—is routinely used to fix (or harden) tissue. Formaldehyde (regularly used as human embalming fluid) is a dangerous chemical and should not be inhaled or allowed to come in contact with human skin. Because formalin is acidic (about pH 5), prolonged immersion of specimens can cause long-term deterioration, such as decalcification of bones. Methods to buffer the acidity of formalin with borax have recently come into question (Hughes & Cosgrove 1990), because these solutions lose their buffering capacity over time. Fortunately, this is only a serious problem for specimens fixed and preserved continuously in formalin. Formaldehyde gas can be soaked out of hardened specimens easily and replaced with less toxic ethanol or isopropanol (usually a 70% by volume solution) for long-term storage. In addition to preventing the subsequent growth of bacteria and fungi, these alcohols allow relatively safe handling and examination of specimens. Preservation in consumable spirits can be effective in an emergency.

Reptile skin retards penetration of formalin, and large specimens must be either cut open and immersed in, or injected with, preservatives. Injection of formalin with syringes and hypodermic needles is most effective, but also hazardous. Insulin syringes with fixed, fine needles are ideal for injecting preservative into the limbs and tails of very small lizards and snakes, and for extracting blood from living specimens (see below). For large animals, with accumulations of fat, it is essential to expose the fatty tissues to formalin without delay, to prevent rapid deterioration. We recommend glass rather than plastic syringes, although they are more expensive. Only Luerlock syringes and needles should be used to prevent the two from separating under pressure of injection. The resulting back-spray almost invariably is directed into the face and eyes of the preparator. Even with Leurlock materials, goggles and rubber gloves are essential.

Whenever possible, specimens should be fixed in positions that facilitate the examination and measurement of body parts (Cogger 1992), particularly where specimens are to be used in studies involving accurate measurements (Fig. 14.4). Consideration must also be given to the size and shape of storage containers available to hold specimens, for example, lizards with long tails may not fit into available bottles, unless the tail is bent into a U-shape during preservation.

Some collectors inject formalin into the base of the tail of male lizards and snakes, to evert one or both hemipenes. The shape and ornamentation of these structures provide additional characters for study. Unless these are tied off at

Figure 14.4 A tray of preserved specimens, illustrating appropriate positions for fixation. Note incisions to promote penetration of fixative and the space-saving which results from coiling the body or flexing the tail. [Photo by T.D. Schwaner]

their bases with a piece of thread, they may subsequently retract. Similarly, turtle necks often must be stretched during the hardening process to prevent retraction of the head. A wad of paper or similar material should be placed in the mouths of specimens to preserve the jaws in an open position, for later examination of the teeth and palate.

Skeletal Preparation

The preservation of skeletal materials usually involves three stages of preparation—maceration, the use of dermestid beetles or meal worms (*Tenebrio*), and alizarin staining. Maceration is a slow process in which, initially, the specimen is skinned, gutted and most of the muscle is cut away. The carcass is placed in water until the remaining flesh is softened so the bones can be picked or brushed cleaned. With care, whole, articulated skeletons can be prepared by this method. Beetles and meal worms can eat the remaining bits of flesh on a prepared carcass. So efficient are these insects that the bones require only a degreasing process to remove odour and prevent the attraction of other insects to the stored collections. However, each specimen must be closely monitored or the efficient beetles will disarticulate the skeletons.

Alizarin staining (for example, Webber 1978b; Zug & Crombie 1970) involves a process that clears the muscle tissue of a skinned, eviscerated specimen using solutions of potassium hydroxide and the enzyme, pepsin. Once cleared, the specimen is placed in a bath of the dye alizarin red which has an affinity for bone. More recent preparations also use green or blue stains that are specific for cartilage (Hanken & Wassersug 1981). The final, cleared and stained specimens are stored in glycerin to which a small amount of thymol has been added to retard fungal growth (see Simmons 1987). So specific are these stains that each bone and articulating cartilage of even the smallest specimens is colourfully and vividly displayed when viewed under a dissecting microscope.

Comprehensive skeletal collections require many hours of preparation and every effort should be made to deposit such collections in museums so that they are readily available for comparisons of recent and fossilised bones.

Chromosomes

The number, shape and internal structure of chromosomes are important characters in systematic biology and have been used extensively to determine the status and relationships of Australian reptiles (see King 1985, and references therein). Chromosome preparations can be obtained from various somatic and reproductive tissues, or from cultured reptilian blood cells (particularly white cells) treated with colchicine to inhibit the formation of a mitotic spindle. Freshly treated cells are then fixed with an ice-cold mixture of three parts ethanol or methanol and one part glacial acetic acid, for long-term storage and handling (see Sessions 1990). Sessions (1990) provides an exhaustive discussion of chromosome preparations including protocols for preparing chromosomal materials in the field. Baker, Bull & Mengden (1971) offer alternative suggestions.

Viable Proteins

Immuno-electrophoresis, immunodiffusion on trefoil ouchterlony plates and microcomplement fixation (MC'F) are techniques that have been used to test the relationships of groups of Australian reptile species using antigen-antibody reactions (Baverstock & Schwaner 1985). These methods require purified albumin or transferrin proteins in blood serum. Adequate amounts of these proteins can be obtained from 1 to 2 ml of whole blood, although larger amounts enable samples to be taken. We have found that blood can be drawn quickly from the heart of a medium to large lizard or snake, using an insulin syringe with a fixed needle (Fig. 14.5). The head of a snake is grasped as described above and held with the back of its neck and upper body resting belly-up on the inner forearm of the holder. The rest of the snake's body is held firmly by an assistant. Careful observation will reveal the position of the beating heart. A needle is inserted quickly under (not through) a ventral plate, two or three scutes below the heart. If the tip of the ventricle is punctured, blood should flow freely into the syringe with gentle retraction of the plunger.

Figure 14.5 Extraction of blood from a live tiger snake *Notechis scutatus* by heart puncture, released and subsequently recaptured, on Kangaroo Island, South Australia. [Photo by M. McKelvey]

In large lizards the needle is inserted through the arm pit, transversely to the plane of the body, to reach the pericardial sac. Some workers are critical of these methods, although we have used them on hundreds of live snakes and lizards without causing the death of a single individual. Nevertheless, alternative methods, such as drawing blood from the caudal sinus, can be used, but this definitely causes discomfort to the animal and care must be taken to hold firmly onto the tail and body during the procedure.

Samples of various tissues (for example, blood, heart, liver, stomach, intestine, kidney and muscle) are essential for genetic studies using electrophoresis of proteins. These tissues must be frozen immediately and maintained at very low temperatures (<-60°C), to preserve proteins for both short-term analysis and long-term storage (Figs 14.6, 14.7). In the field, tissues may be frozen using either 'dry ice' (*i.e.* frozen CO_2 at about -60°C), or liquid nitrogen (at about -196°C). Special containers are necessary for transporting liquid nitrogen. Those that hold spillable liquids are not usually allowed on commercial aircraft. However, Taylor-Wharton Company (Indianapolis Indiana, United States of America) manufactures a 'dry shipper', in 3 or 5 litre sizes, that absorbs liquid nitrogen into a special material located between the chamber and inner shell of the container. Once this material is saturated, excess liquid nitrogen can be poured off. The absorbed liquid keeps samples at ultracold temperatures for days or weeks (depending on the size of the container). These flasks can be carried as baggage on commercial aircraft, but special clearance may be necessary on overseas flights.

Figure 14.6 Blood samples can be separated into plasma and the cellular fraction by centrifugation. Robust, hand-operated devices can be very effective in field conditions. [Photo by T.D. Schwaner]

Figure 14.7 Storage of tissues in an ultra-cold storage unit at the South Australian Museum. Such units maintain sufficiently low temperatures to preserve proteins for both short-term analysis and long-term storage.
[Photo by Adrienne Edwards]

The relative positions of internal organs are good characters for systematic study. When taking tissues make small, clean incisions. Inflict minimal damage to skin, bone and unwanted organs. Take only portions of organs—for example, the ventricle of the heart, the middle section of the liver (for snakes), stomach or intestines, one kidney, muscle from one hind leg or one side of the body. Consistently dissect the same side of the body for each specimen. Package each tissue separately, but store tissues from the same individual together, or mark each package in such a way as to indicate which specimen each came from (see Dessauer & Hafner 1984).

Nucleic Acids

The number of potential characters for systematic studies of reptiles has been vastly increased by methods that determine the sequence of nucleic acids in their DNA (Werman, Springer & Britten 1990; Dowling, Moritz & Palmer 1990; Hillis, Larson, Davis & Zimmer 1990). In addition, the polymerase chain reaction (PCR) is an exciting new technique for both molecular biologists and curators of traditional museum collections, because fragments of DNA extracted from alcohol or even formalin-fixed specimens can be multiplied (i.e. amplified), overnight, to amounts sufficient for DNA sequencing (Innis, Gelfand, Sninsky & White 1990). These methods finally solve the major drawbacks of previous molecular techniques—namely, the need for large amounts of material from several (often very small) specimens, with the frequent (and increasing) requirement not to kill the animals in the process. As these new methods develop, properly preserved collections of reptiles will become even more valuable than before.