



FAUNA *of* AUSTRALIA



3. COLLECTION AND PRESERVATION OF THE ANURA

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INTRODUCTION

The collection and preservation of frogs is an important component of several research disciplines. Specimens are required for taxonomic research and phylogenetic studies. Increasingly, pharmacological studies show that animals, as well as plants, are storehouses of potentially beneficial substances. Frogs are sensitive environmental indicators and records of their occurrence, abundance and disappearance are of prime importance for environmental monitoring purposes. Voucher specimens may be vital in surveys, maintaining some of the information lost through habitat destruction.

Only the minimum numbers necessary to achieve scientific objectives should be collected. In particular, species with restricted distributions, small population sizes and low recruitment should be collected sparingly to minimise impact.

CAPTURE

Frog collecting is often fortuitous because it is heavily weather dependent, especially for fossorial species which only emerge after heavy rain. Although aestivation sites of cool temperate species may sometimes be located (McDonald & Davies 1990), the breeding season is the most effective time to collect frogs. A powerful torch is essential for this predominantly nocturnal activity. A head torch frees both hands and permits recognition of eye shine in many species, while a hand-held torch generally is more powerful.

Calling by male frogs to advertise their presence during the mating season tends to bias collections towards males. As direction but not distance from which calls emanate can be judged quite readily, triangulation is the most effective way of finding calling frogs, despite the ventriloquism of many species. Two or three persons stand 2 to 3 m apart in the general vicinity of the calling frog, identify the direction of the call, and aim their torches at its source. The frog will be located close to where the beams intersect. During this process, the frog may stop calling, but usually it will resume if the observers stand quietly.

Foraging frogs and females homing toward calling males can be located by torchlight near free water or choruses of frogs. It is important to establish landmarks prior to such searches, as they can be extremely disorientating.

Roads, especially sealed ones, can be fruitful collecting areas, particularly after rain. With some experience, species can be recognised in the headlights of a vehicle travelling up to 40 km/hr by traits of posture and gait. This 'road running' technique is a valuable form of survey, especially combined with stationary periods to identify species by call.

By day, inactive frogs can be collected in damp places such as vegetation around pools, under rocks and logs, in crevices of tree trunks and in caves. Frog spawn and tadpoles are usually collected by day using a dip net or a soup strainer; terrestrial spawn are collected by hand.

Collectors must ensure that their specimens are not unduly stressed, and consider the sensitivity of frogs to heat and their need for constant moisture at all times. For short periods only, adult frogs, spawn and tadpoles should be placed in inflated and moistened polythene bags (305 mm x 445 mm x 50 mm). The number of frogs in a single bag should be limited, according to their size. Often it is inadvisable to mix frogs of different sizes within a bag because some species are cannibalistic or will eat smaller species held with them. Under the stress of captivity and confinement, some species also release skin secretions which are toxic to other frogs.

Moist calico bags are excellent repositories for frogs, particularly if they are to be kept alive for long periods. Loose cotton threads should be removed from the inside of the bag to avoid entanglement of limbs or digits.

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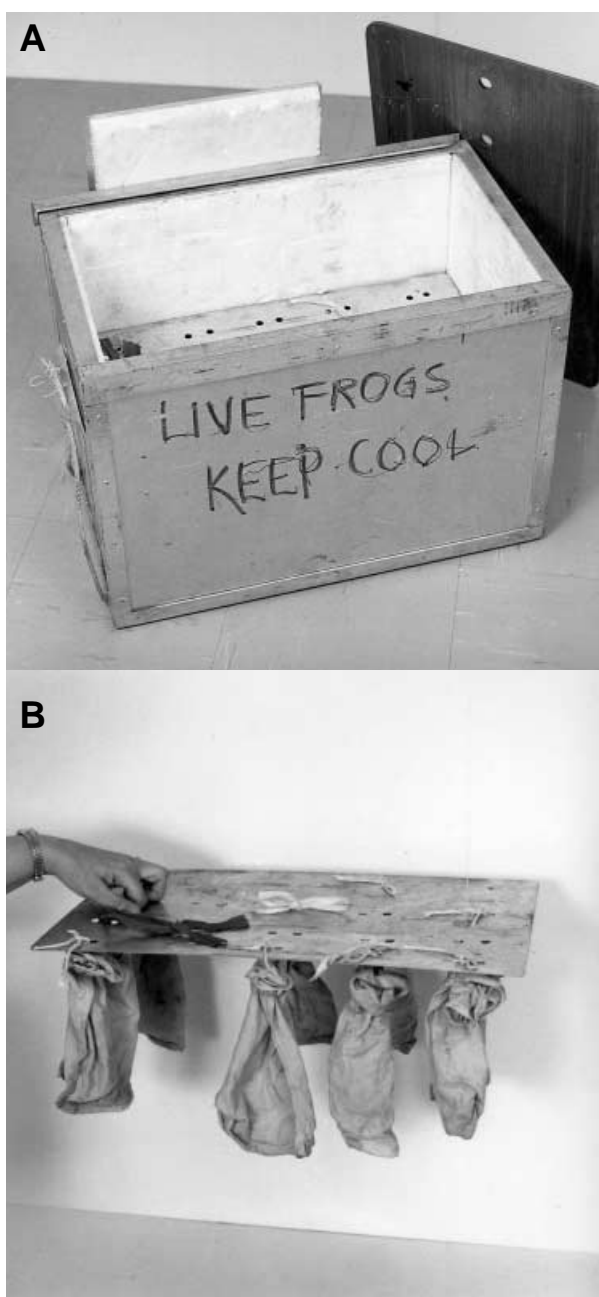


Figure 3.1 Collecting box for frogs. **A**, aluminium container lined with polystyrene. **B**, a narrow lip at mid-depth supports a perforated aluminium shelf from which calico bags are suspended in the lower section. Tadpoles, or frogs in calico or inflated bags can be transported in the upper section. [Photos © P. Kempster]

Polythene or calico bags should be kept in a cool place; a polystyrene cooler is ideal. Calico bags should be suspended within the container to enable plenty of air flow if they are to be transported over long distances. Over long periods, frogs survive less successfully in inflated polythene bags. Dry polythene bags are best, as evaporation from the frogs maintains the required humidity; additional water becomes contaminated very easily and putrefies. Bags must be checked regularly if kept in the field for any length of time, to ensure that they remain moist, inflated and cool, and to remove fatalities before they decompose and cause further deaths.

Separation of sexes will prevent spawning. If spawning is required, males and females (amplectant or not) should be placed together in inflated polythene bags with enough water for spawning to occur. For foam nesting species, the water depth should be sufficient for the female to paddle her forelimbs during foam formation (Tyler & Davies 1979c). Water required by terrestrial ovipositors for deposition of eggs should be replaced with damp vegetation after laying. Bags should be kept upright during oviposition because eggs laid out of water are generally infertile or are hard to retrieve.

As a minimum requirement, all specimens must be identified by a name, and the date and locality of collection. Behavioural and ecological data should be recorded whenever possible. These include whether the frogs called, their calling location, whether other species called in synchrony, and the nature of the aquatic habitat (permanent, ephemeral, static or moving).

Frogs are totally protected in most States, in all National Parks and State Forests and partially protected in other areas, so permits to collect and, in some cases, to export between States are required. As regulations vary from State to State, it is essential to find out from the appropriate State or Federal authorities the nature

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of the restrictions applicable in any area and the types of permits required before collecting. Export of live or preserved frogs from Australia is subject to Federal control, irrespective of the origin of the fauna. Some species, including the gastric brooding frogs *Rheobatrachus silus* and *R. vitellinus*, are prohibited exports. Comments on pertinent State and Federal legislation and other sources of information for collectors are provided in Chapter 14.

PRESERVATION AND STORAGE

Frogs die in a relaxed position, following gentle anaesthesia, when placed in enough 3% chloral hydrate (1% for small frogs) solution to allow absorption through the ventral skin. The dead specimens should be placed in a tray or baking dish on paper towelling moistened with 3% formalin (to facilitate subsequent histology). The fingers and toes should be arranged to enable maximal information to be obtained. The specimens should be covered with another layer of moistened paper towelling and the tray sealed in a polythene bag to prevent desiccation. After about an hour *rigor mortis* should have set in and identification labels can be attached at the knee. Labels must be waterproof and written in pencil or waterproof ink.

Specimens should be stored in 3% formalin or 65% ethanol. Formalin is convenient in the field as it can be transported in concentrated form for dilution with water as required. Before transport, it can be drained and replaced with formalin-soaked cotton wool to reduce weight and prevent possible spillage. In the laboratory, specimens should be transferred to 65% ethanol. Specimens should be stored in glass jars that are large enough not to distort the spread limbs.

Tadpoles should be killed in 3% chloral hydrate and preserved in Tyler's (1962) fixative (10 ml concentrated formalin, 4 ml 10% aqueous calcium chloride, 0.2 g cobalt nitrate, 90 ml water). This fixative prevents degradation of the outer epithelium and thus maintains specimens suitable for histology. Alternative preservation techniques are discussed by Pisani (1973).



Figure 3.2 Frogs laid out on formalin dampened paper. The limbs are positioned for maximal information. [Photo ©P. Kempster]

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Snap freezing in liquid nitrogen or dry ice and long term storage at -80°C is the most effective method for preserving the widest variety of tissue constituents for biochemical and karyological analyses. Dessauer, Cole & Hafner (1990) provide information on collection and preservation of material for various biochemical analyses. If snap freezing is not possible under field conditions, and material cannot be transported live back to the laboratory, an aqueous solution of 2% 2-phenoxyethanol preserves many enzymes for at least three weeks (Nakanishi, Wilson, Nolan, Gorman & Bailey 1969). Plasma albumins for microcomplement fixation studies can be preserved in this solution at room temperature for up to a year.

LABORATORY CULTURE

Amphibians can be reared and housed successfully in a laboratory, in conditions similar to their natural habitat requirements. Tropical species should be kept at 25 to 30°C while temperate species can be housed satisfactorily at 20°C. Daylight fluorescent tubes should be installed to ensure adequate synthesis of keratin if natural light is unavailable.

Tadpoles can be reared in glass or plastic containers, in aerated pond water or tap water which has been suitably 'aged' by thorough aeration for 24 hr before use. Hyloid tadpoles can be reared successfully on a diet of lightly boiled or frozen lettuce or spinach leaves, whilst many myobatrachids require a high protein supplement such as breakfast cereal or fish food. It is important not to overfeed tadpoles as this fouls the water, which should be changed before it becomes tainted; usually this means a daily change of water for older tadpoles. As metamorphosis approaches, an accessible surface extending above the water should be provided for froglets to leave the water.

Newly metamorphosed frogs require large amounts of small food items. Vinegar flies *Drosophila melanogaster*, especially flightless forms, are particularly suitable. Slightly larger froglets can eat small mealworms, *Tenebrio molitor*, or cockroach nymphs. Rearing froglets from metamorphosis to a size at which a varied diet of larger prey items can be provided is difficult, and mortality at this stage is high.

Adult frogs keep well in an aquarium with a pond at one end and land at the other. Moss, rocks, leaf litter and other vegetation provide ideal shelter, and should allow ground dwelling species to enter or leave the pond easily. A light wooden frame to support fine cloth or wire mesh, with foam glued along two inside edges, will deter the escape of large, actively-climbing frogs and prey items alike.

Frogs generally require moving prey. Most species thrive on a diet of mealworms varied with other insects. Dusting mealworms with powdered vitamins will improve an unvaried diet. Frogs of different sizes should not be mixed in an aquarium, to prevent some frogs eating others and starvation of smaller ones through competition for food. However, ground dwelling and tree frogs of similar sizes can be housed together.

Redleg is a contagious bacterial disease of captive amphibians that is difficult to eradicate (Banks 1980). Tyler (1987b) noted an oedema of unknown origin which appears to be incurable.