

Frogs and Toads as Experimental Animals

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Fact Sheet

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Introduction

Frogs and toads were used extensively for experimental purposes for several decades, but their current use is more as subjects for dissection in tertiary teaching institutions, than for investigations in the fields of physiology and pharmacology. Given recent concerns about the decline or total disappearance of some species of frogs, it is likely that research will focus more upon use of the introduced toad: the Cane Toad *Bufo marinus* and the introduced African Clawed Frog *Xenopus laevis*.

Historically, Australian amphibians were perhaps first used extensively as the subjects for the study of helminth parasites by T. Harvey Johnston and his colleagues at the University of Adelaide. Following the demonstration by Bhaduri and Bardhan (1949) that a simple diagnostic test for human pregnancy involved the injection of a sample of the patient's urine into a South American frog, *Bufo marinus* was found to behave similarly, and its use in Australia for this purpose rapidly ran into many thousands each year. Of 16,000 imported into South Australia in 1966, the majority were for pregnancy testing purposes. In university and other institutional research, the isolated sciatic - gastrocnemius muscle preparation and the isolated rectus abdominis muscle were used routinely for demonstration of nerve-muscle transmission, and for acetylcholine assays respectively. In recent years *Bufo marinus* has

found a new role as a subject for toxicological studies of environmental pollutants. It follows that there remains a need to consider the available information on the housing, care and maintenance of frogs and toads in captivity.

Sources

The collection and interstate movement of native frogs in most States and the Territories is subject to various legislative laws and regulations. Information needs to be obtained from the relevant National Parks and Wildlife Service or its equivalent. Similarly, the ambit of animal ethics committees includes the review of experimental procedures on frogs and toads, and appropriate research permits are required.

To my knowledge there is only one commercial source of native species: the Amphibian Research Centre in outer Melbourne. A few native species can be purchased from pet shops, but their availability is often uncertain and, unless they can be guaranteed to have been obtained legally, these sources of supply should not be used. Cane Toads can be obtained from various suppliers in Queensland. Because of the pest status of this species, importation from suppliers is regulated, and a permit is required from the vertebrate pest authority in the State to which they are being imported. Colonies of the African Clawed Frog are maintained in a number of institutions. Like the Cane Toad, this species has the potential to become established

in the wild if released. Accordingly, it is subject to maintenance and transfer controls, and is a prohibited species in Western Australia.

Housing

Because of the assumption that few native species are likely to be used as experimental animals (other than perhaps as a source of eggs), I will confine my observations to one common and widespread native species, the Marbled Frog, *Limnodynastes tasmaniensis* and the two exotic species mentioned above. In the event that none of these is suitable for a particular purpose, advice on other species should be sought from amphibian experts at major zoos or museums.

(a) *Limnodynastes tasmaniensis*

This species has a snout-vent length of up to 50 mm, and a group of up to 12 individuals can be maintained in a glass aquarium with a floor area of 50 cm x 30 cm and a height of 30 cm. The species lives around the edges of pools, lakes and streams and shelters at the base of dense vegetation or beneath rocks or debris during the day. It follows that the aquarium should provide land, water and cover.

The substratum most favoured in our laboratory is bricks of compressed, chopped and dehydrated coconut fibre. When placed in a bucket of water it expands to form a soft, moist material. Occasionally *L. tasmaniensis* will attempt to climb or jump out, and an aquarium lid is essential. We use a custom-made wooden lid that slips over the glass and rests upon the upper rim. The frame is covered with a fine, strong, open-weave cotton material similar to that used for mosquito nets.

A larger and more elaborate fibreglass tank, featuring a flowing stream, was developed at the University of Adelaide, and is described by Chapman (1987).

(b) *Xenopus laevis*

This species is totally aquatic. Because there are two books providing all data relevant to its care and maintenance it is sufficient to refer to them (Deuchar, 1975; Tinsley and Kobel, 1996). The only way to obtain this species is from a laboratory maintaining a colony and, hence, information about housing can be obtained from the supplier prior to establishing a new colony.

(c) *Bufo marinus*

There was a time when Cane Toads were housed in large zinc chambers within animal houses. No food was provided and the only shelter was in the form of wet sacks thrown upon the floor. Happily it is now appreciated that, despite their pest status, they require food and warmth if they are to maintain optimal condition and the standard of care must satisfy modern ethical criteria.

There are two uses for which Cane Toads are likely to be obtained: as a source of tissues or for other purposes that require that the animal be sacrificed, or as a source of fertile eggs. Holding tanks of glass or fibreglass are ideal. Access to water is essential - heavy china drinking bowls are particularly suitable because they cannot be overturned. The housing of Cane Toads intended to be used for breeding purposes is described below.

Environmental conditions

Light quality

If the room in which the animals are to be housed lacks direct light, it is imperative that the fluorescent lighting be of 'daylight' quality. The health of the animals (and the rearing of healthy, normal tadpoles) requires exposure to low levels of ultraviolet light.

Air quality

In an animal house, the only major concern is to ensure that any air-conditioning is separate from that used for rats, mice, rabbits and other mammals, because amphibians are extremely sensitive to ammonia and will not survive exposure to it.

Temperature

As a convenient rule, frogs and toads from northern Australia should be maintained at 30°C, and those from southern Australia at 20°C. In northern Australia tadpoles and eggs commonly are found in water with temperatures above 40°C and on one occasion at 45°C (Tyler, 1994). Nevertheless, the attributes to survive at such temperatures are species-specific and care should be taken regarding their exposure to such temperatures even for short periods.

Food

The larvae of the beetle *Tenebrio molitor* (better known as mealworms) are by far the best staple diet for frogs. Toads are equally partial, but their extreme dietary intake can make this food an expensive maintenance item. Mealworms can be purchased in amounts as little as 100g from pet shops, for they are also fed to lizards, turtles and insect-feeding birds and marsupials. The mealworms are usually supplied in plastic containers of dry bran to absorb moisture. The development of larvae can be halted by storing them in a refrigerator at 4°C. If the quantity of mealworms required places financial stress upon the research budget, a mealworm breeding colony can be established. Details can be obtained from pet clubs or caged bird fanciers and are described in Tyler (1997).

Alternative food sources are crickets and native cockroaches, which can be purchased in various sizes.

Anaesthesia

There are relatively few instances in which there are a need to anaesthetise frogs, and relatively few options to achieve that state. By far the best documented general anaesthetic is the cocaine isomer MS-222 (tricaine methanesulfonate (TMS) ethyl 14 - aminobenzoate), rejected for human use (as a local anaesthetic) because of its low solubility in water. It is now available from Ruth Consolidated Industries of Annandale, NSW. One of the major attributes of MS-222 is the simple and non-invasive method of delivery. All that is required is to place the amphibian in a

2% aqueous solution, no more than 5 mm deep. The anaesthetic is absorbed directly through the skin of the ventral surface of the body and limbs. It is preferable to make up a fresh solution for each use, because there is significant loss of activity after a few days.

The depth of anaesthesia can be determined in precisely the same manner as for small mammals: by compression at an inter-phalangeal joint. The duration of induction will vary with the size, body mass and skin thickness of the species, combined with body temperature and hence, metabolic activity. Elimination of the anaesthetic is brought about by exposing the animal to a stream of cold water beneath a tap. Fingerling trout have been anaesthetised for up to eight hours, but shorter periods are recommended.

Fluothane (ICI) has been used as a general anaesthetic for Cane Toads (Taylor *et al.*, 1985). A few millilitres are poured upon a wad of cotton wool in a sealed, five litre capacity glass jar beside the toad. Loss of consciousness is rapid, and anaesthesia is maintained for three to five minutes following removal from the container. The depth of anaesthesia is sufficient for minor surgical procedures.

Anholt *et al.* (1998) described the use of a 0.02% solution of MS-222 to anaesthetise tadpoles. Immobility was achieved within four minutes, with recovery after thirty minutes.

Euthanasia

The prospect of using decapitation as a means of euthanasing frogs is a deterrent to those contemplating experimental biology. Despite its speed and efficacy it is considered barbaric by many researchers and by those opposed to the use of animals for experimental purposes.

A humane euthanasing agent is a 3% aqueous solution of chloral hydrate. As with the use of MS-222 as a general anaesthetic, the frog is placed in a container with the solution covering its floor to a depth of no more than 5 mm. Even

with a tree frog, which may climb out of the solution, death follows within a few minutes and the animal is in a relaxed state.

Deep-freezing is used by many to kill cane toads. It is effective, but whether or not there is potential for this practice to cause suffering is difficult to establish.

Pithing (decerebration followed by spinalisation) is more readily demonstrated than described. The diameter and length of the pithing rod and the shape of the tip are crucially important if the procedure is to be undertaken humanely.

I am dubious that the necessary skill and confidence to undertake pithing can be derived other than by video or personal demonstration. The accompanying notes are included simply because this facts sheet would be incomplete without reference to the procedure.

The pithing rod needs to be of stainless steel or any non-ferrous metal of similar rigidity. Kirschner wires of vanadium are ideal. The diameter must be narrower than the diameter of the neural arches through which it must eventually pass. A diameter of 2 mm is my preference.

Decerebration is made possible by the simple form of the first cervical vertebra. The animal needs to be held firmly in the left hand, and the head ventriflexed gently with the forefinger. By this means a non-bony gap is created between the first vertebra and the occipital region of the cranium.

The pithing rod needs to be held near the end of the shaft and inserted forcefully into the brain by rotating it over the forefinger.

Very slowly the pithing rod should be withdrawn to the position at which it rests upon the superior border of the neural arch, and therefore is in a position in which it can be rotated posteriorly and pushed posteriorly (spinalisation) through the neural arch. As soon

as the pithing rod has entered the posterior portion of the spinal canal the operator should turn his or her head to one side, because there can be a massive sympathetic stimulation of the parotoid glands, discharging the toxic contents in a fine spray for a distance of one metre or more. Contact of the secretions with the eyes will cause excruciating pain. The wearing of protective glasses is essential.

Breeding induction

Traditionally, breeding has been induced by the subcutaneous injection of a suspension of crushed toad pituitary glands. It is far simpler to use luteinising hormone releasing hormone (LHRH) produced by Sigma. Because of high quarantine and handling charges in Australia the price for 1 mg has increased greatly. Fortunately, a synthetic form of LHRH ('Lucrine') is used in fertility clinics. Inject 0.05 ml, which equates to 125 µg subcutaneously (s/c) into the male and female, beneath the dorsal skin. Because the skin is so tough in *Bufo marinus*, a 21 gauge needle is needed. The onset of amplexus is rapid. It is commonly one hour after administration but on one occasion the male clasped the female in less than five minutes.

Tadpole care

The chlorine levels in tap water are high enough to kill tadpoles. To eliminate the chlorine it should be allowed to stand in a bucket for 24 hours or passed through a carbon filter. Chloraminated water requires chemical treatment before it is suitable.

Aeration is essential to permit high numbers of tadpoles to survive. When the front legs emerge the tadpoles should be removed and transferred to a separate aquarium with shallow water and stones in readiness for their change in diet and habitat.

I favour boiled lettuce leaves for food, but it is essential to obtain them from an 'organic' source to ensure that they have not been exposed

to pesticides. They should be boiled in a stainless steel vessel to eliminate heavy metal take-up. I purchase 50 lettuces at a time from the grower, boil the leaves, divide them into daily rations and then deep-freeze them in small plastic bags to provide a constant source.

Blood collection

The only reliable method of blood collection is by cardiac puncture. In *Bufo marinus* the ventricle is located near the xiphisternum. I am uncertain whether the position varies to any significant degree in other species. Accordingly, dissection of a freshly sacrificed individual to familiarise the operator with the anatomy should be undertaken prior to the use of this procedure.

Marking

Because frogs shed (and usually eat) their entire skin approximately once each week, there is no simple method of marking individuals externally. I have a personal aversion of toe clipping - removing one or more phalanges to identify individuals. Tattooing or hotwire branding is more humane.

Drug administration

Amphibians have a unique lymphatic system consisting of a series of subcutaneous sacs that drain into pulsatile lymph hearts. The number of sacs and the position of the septa bounding them vary from species to species (Carter, 1979). The dorsal sac extends from the base of the cranium to the end of the body. A drug injected s/c reaches the blood stream within seconds of administration. Apparently unique to *Bufo* is the presence of numerous adhesions of the dorsal skin to the underlying musculature.

Intraperitoneal injection is unreliable because of the often vast size of the bladder (and hence the risk of injecting into it), and the presence of nephrostomes on the surface of the kidney, which take up fluid from the body cavity and

excrete it, so eliminating at least part of any drug administered by that route.

I am unaware of the intramuscular route being used, probably because of the ease of subcutaneous injection.

Diseases

Amongst captive frogs the most common disease is 'Red Leg' caused by the bacterium *Aeromonas hydrophilia* and characterised by inflammation of capillary vessels upon the limbs. Exposure to a 1% aqueous solution of copper sulphate is usually an effective treatment.

Over the past decade a major fungal infection of skin termed chytridiomycosis ('chytrid disease') has proved a severe disease in captive frogs and in the wild. The disease and its impact was reported by Berger *et al.* (1998) and Berger and Speare (1998) in *ANZCCART News* with the infective agent named *Batrachochytrium dendrobatidis* by Longcore *et al.* (1999). How the motile spores invade the dermis is not known.

There are three reviews of frog diseases:

Wright and Whitaker (2001), Reichenbach-Klinke and Elkan (1965) and Raphael (1993). The first named review is the most complete in its scope.

Parasites

Frogs have the capacity to carry extremely high parasite loads without any evident deleterious effects. As a resource for parasitological studies, there has been a number of significant papers and reviews of parasite groups over the past two decades. The majority of these publications have concentrated on the identification of the parasites and providing primary and secondary host lists. For example, Edmonds (1989) reviewed the Acanthocephala for all vertebrate hosts, whilst Barton (1994) provided a check list of all helminth parasites of Australian frogs.

Greater detail of the parasites is provided in the studies of protozoans (Delvinquier, 1986; 1989), trypanosomes (Delvinquier and Freeland, 1989) and opalinids (Delvinquier, 1987).

Myxidium immersum has been linked to hepatitis in *Litoria caerulea* by Hill *et al.* (1997), but experimental infection has to date proved unsuccessful.

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