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NSW  
NATIONAL  
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WILDLIFE  
SERVICE

**NSW NATIONAL PARKS AND WILDLIFE SERVICE**

*Threatened Species Management*

## **Information Circular No. 6**

**Hygiene Protocol for the Control of Disease in Frogs**

**May 2000**

## **Acknowledgments**

NSW National Parks and Wildlife Service Declining Frog Working Group who recommended the preparation and provided input into the development of this strategy.

Ross Wellington (NPWS) is the principal author of this document with assistance from Ron Haering (NPWS).

Lee Berger, Arthur White, Julie Ravallion, Graham Pyke, Jeff Hardy and Jack Baker for their advice and/or technical review.

This hygiene protocol is an adaptation of the Declining Amphibian Population Task Force (DAPTF) Fieldwork Code of Practice and the recommendations of Speare et al. (1999).

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# 1. Introduction

This information circular outlines measures to:

- Prevent or reduce disease causing pathogens being transferred within and between wild populations of frogs.
- Ensure captive frogs are not infected prior to release.
- Deal safely with unintentionally transported frogs.
- Assist with the proper identification and management of sick and dead frogs in the wild.

## 1.1 Who should read this document?

This protocol is intended for use by all researchers, wildlife consultants, fauna surveyors and students undertaking frog field-work. In addition, the protocol should be read by National Parks and Wildlife Service (NPWS) personnel, frog keepers, wildlife rescue and carer organisations, herpetological/frog interest groups/societies, fauna park/zoo operators/workers and other individuals who regularly deal with or are likely to encounter frogs.

This protocol outlines the expectations of the NPWS regarding precautionary procedures to be employed when working with frog populations. The intention is to promote implementation of hygiene procedures by all individuals working with frogs. New licences and licence renewals will be conditional upon incorporation of the protocol. The NPWS recognises that some variation from the protocol may be appropriate for particular research and frog handling activities. Such variation proposals should accompany any licence application or renewal to the NPWS.

## 1.2 Background

The apparent decline of frogs, including extinctions of species and local populations, has attracted increased international and national concern. Many potential causes for frog declines have been proposed (eg see Pechmann et al., 1991; Ferrero and Bergin, 1993; Pechmann and Wilbur, 1994; Pounds and Crump, 1994; Pounds et al., 1997). However, the patterns of decline at many locations suggest that epidemic disease maybe the cause (Richards et al., 1993; Laurance et al., 1996; Alford and Richards, 1997). Recent research has implicated a water-borne fungal pathogen *Batrachochytrium* as the likely specific causative agent in many of these declines both in Australia and elsewhere (Berger et al., 1998; 1999). This agent is commonly known as the Chytrid fungus and is responsible for the disease Chytridiomycosis (Berger et al., 1999).

The NPWS established a Declining Frog Working Group (DFWG) in 1998 to develop specific frog conservation strategies and initiatives in NSW. An important issue identified by the DFWG, was the need for a hygiene protocol for field workers to minimise the spread of the Chytrid fungus and other pathogens, between sites where frogs occur and between individual frogs and/or tadpoles.

## 1.3 Objectives

The objectives of the hygiene protocol are to:

- Recommend best-practice procedures for NPWS personnel, researchers, consultants and other frog enthusiasts or individuals who handle frogs.
- Suggest workable strategies for those regularly working in the field with frogs or conducting fieldwork activities in wetlands and other aquatic environments where there is the potential for spreading pathogens such as the Chytrid fungus.
- Provide background information and guidance to people who provide advice or supervise frog related activities.
- Provide standard licence conditions for workers engaged in frog related activities.
- Inform Animal Care and Ethics Committees (ACEC) for their consideration when granting research approvals.

# 2. Site Hygiene Management

Individuals studying frogs often travel and collect samples of frogs from multiple sites. Some frog populations can be particularly sensitive the introduction of infectious pathogens such as the Chytrid

fungus. Also, the arrangement of frog populations in the landscape may make them particularly vulnerable to transmission of infectious pathogens. Therefore, it is important that frog workers recognise the boundaries between sites and undertake measures which reduce the likelihood of spreading infection.

Where critically endangered species or populations of particular risk are known to occur, this protocol should be applied over very short distances ie a single site may need to be subdivided and treated as separate sites.

A checklist of procedures is provided in Appendix 1.

## **2.1 Defining a Site**

Defining the boundary of a site maybe problematic. In some places, the boundary between sites will be obvious but in others, less so. Undertaking work at a number of sites or conducting routine monitoring at a series of sites within walking distance creates obvious difficulties with boundary definitions. It is likely that defining the boundary between sites will differ among localities. It may be that a natural or constructed feature forms a logical indicator of a site boundary eg a road/track, a large body of water such as a river or the sea, a marked habitat change or a catchment boundary.

***As a guiding principle, each individual waterbody should be considered a separate site.***

When working along a river or stream or around a wetland or a series of interconnecting ponds it is reasonable, in most instances, to treat such examples as a single site for the purposes of this protocol.

Where a stream consists of a series of distinctive tributaries or sub-catchments or where there is an obvious break or division then they should be treated as separate sites.

## **2.2 On-site Hygiene**

When travelling from site to site it is recommended that the following hygiene precautions be undertaken to minimise the transfer of disease from footwear, equipment and/or vehicles.

### ***Footwear***

***Footwear must be thoroughly cleaned and disinfected at the commencement of fieldwork and between each sampling site.***

This can be achieved by initially scraping boots clear of mud and standing the soles in a disinfecting solution. The remainder of the boot should be rinsed or sprayed with a disinfecting solution. Disinfecting solutions should be prevented from entering any water bodies.

Rubber boots such as ‘gum boots’ or ‘Wellingtons’ are recommended because of the ease with which they can be cleaned and disinfected.

Several changes of footwear bagged between sites might be a practical alternative to cleaning.

### ***Equipment***

***Equipment such as nets, balances, callipers, bags, scalpels, headlamps, torches, wetsuits and waders etc that are used at one site must be cleaned and disinfected before re-use at another site.***

Disposable items should be used where possible. Non-disposable equipment should be used only once during a particular field exercise and disinfected later or disinfected at the site between uses using procedures outlined in 2.4 below.

## Vehicles

**Where necessary, vehicle tyres should be sprayed/flushed with a disinfecting solution in high-risk areas.**

Transmission of disease from vehicles is unlikely to be a problem. However, if a vehicle is used to traverse a known frog site, which could result in mud and water being transferred to other bodies of water or frog sites, then wheels and tyres should undergo cleaning and disinfection. This should be carried out at a safe distance from water bodies, so that the disinfecting solution can infiltrate soil rather than run-off into a nearby water body.

Spraying with hospital grade 'toilet duck' (active ingredient benzalkonium chloride) is recommended to disinfect car wheels and tyres. 'Toilet Duck' is also suitable for disinfecting footwear.

Cleaning of footwear will prevent the transfer of pathogens from/to vehicle floor and control pedals.

### 2.3 Handling of Frogs in the Field

The spread of pathogenic organisms, such as the Chytrid fungus, may occur as a result of handling frogs.

**Frogs should only be handled when necessary.**

Where handling of frogs is necessary the risk of pathogen transfer should be minimised as follows:

- Hands should be either cleaned and disinfected between samples or a new pair of disposable gloves used for each sample. This may be achieved by commencing with a work area that has a dish containing a disinfecting solution and paper towels.
- A 'one bag – one frog' approach to frog handling should be used especially where several people are working together with one person processing frogs and others doing the collecting. Bags should not be reused.
- A 'one bag – one sample' approach to tadpole sampling should be used. Bags should not be reused.

Toe clipping or Passive Integrated Transponder (PIT) tagging is likely to increase the risk of transmitting disease between frogs due to the possibility of directly introducing pathogens into the frogs' system. This can be minimised by using:

- Disposable sterile instruments
- Instruments disinfected previously and used once
- Instruments disinfected in between each frog

Open wounds from toe clipping and PIT tagging should be sealed with a cyanoacrylate compound such as *Vetbond*<sup>®</sup> to reduce the likelihood of entry of pathogens. The NPWS ACEC further recommends the application of topical anaesthetic *Xylocaine*<sup>®</sup> cream and *Betadine*<sup>®</sup> disinfectant solution before and after any surgical procedure. This should then be followed by the wound sealant.

All used disinfecting solutions, gloves and other disposable items should be stored in a waste container and disposed appropriately at the completion of fieldwork. Disinfecting solutions **must not** come into contact with frogs **or** be permitted to contaminate any water bodies

### 2.4 Disinfection Methods

Disinfecting agents for hands and equipment must be effective against bacteria and both the vegetative and spore stages of fungi. The following agents are recommended:

- Chloramine and Chlorhexidine based products such as *Halamid*<sup>®</sup>, *Halasept*<sup>®</sup> or *Hexifoam*<sup>®</sup> are effective against both bacteria and fungi. These products are suitable for use on hands, footwear, instruments and other equipment. The manufacturers instructions should be followed when preparing these solutions.

- Bleach and alcohol (ethanol or methanol), diluted to appropriate concentrations can be effective against bacteria and fungi. However, these substances may be less practical because of their corrosive and hazardous nature. When using methanol either:
  - immerse in 70% methanol for 30 minutes or
  - dip in 100% methanol then flame for 10 seconds or boil in water for 10 minutes

Some equipment not easily disinfected in these ways can be effectively cleaned using medical standard 70% isopropyl alcohol wipes - *Isowipes*®.

### 3. Captive Frog Hygiene Management

#### 3.1 Housing Frogs and Tadpoles

*Frogs and tadpoles should only be removed from a site when absolutely necessary.*

When it is necessary for frogs or tadpoles to be collected and held for a period of time, the following measures should be undertaken:

- Animals obtained at different sites should be kept isolated from each other and from other captive animals.
- Aquaria set up to hold frogs should not share water, equipment or any filtration system. Splashes of water from adjacent enclosures or drops of water on nets may transfer pathogens between enclosures.
- Prior to housing frogs or tadpoles, ensure that tanks, aquaria and any associated equipment are disinfected.
- Tanks and equipment should be cleaned, disinfected and dried immediately after frogs/tadpoles are removed.

#### 3.2 Tadpole Treatment

In most instances:

*Release to the wild of tadpoles held or bred in captivity should be avoided*

When contemplating a release of captive bred tadpoles for conservation purposes a Translocation Proposal should be submitted to the NPWS and pathological screening for disease should be undertaken (see also NPWS Draft Translocation Policy). Tadpoles can be tested by randomly removing 10 individuals at 6 weeks and again at 2 weeks before anticipated release. Testing could be undertaken by the pathology section at Taronga Zoo, Newcastle University, CSIRO Australian Animal Health Laboratories at Geelong and James Cook University at Townsville. Such an arrangement would need to be negotiated by contacting one of these institutions well before the anticipated release date. (see Appendix 2 for contact details)

The NPWS intends to licence NSW Department of Education and Training (DET) to allow school students and/or teachers to remove tadpoles for classroom life cycle studies. They will be authorised to remove a maximum of 20 individuals from only one location and each school will also require endorsement from DET Animal Care and Ethics Committee and comply with this protocol.

Tadpoles collected for these purposes are to be obtained from the local area of the school and are not to be obtained from NPWS Reserves. As soon as tadpoles have transformed, froglets must be returned to the exact point of capture. Tadpoles from different locations are not to be mixed.

Antifungal cleansing treatments to clear tadpoles of the Chytrid fungus are currently being trialed. In the future, such a treatment may be an added procedure required prior to froglet releases.

### 3.3 Frog Treatment

The rigour with which frogs must be treated to ensure pathogens are not introduced to native populations means that any proposal for the removal of adult frogs (particularly threatened species) from wild populations should be given careful consideration.

When it is essential for frogs to be removed from the wild, the following should apply.

***Individuals to be released should be quarantined for a period of 2 months and monitored for any signs of illness or disease.***

Frogs must not be released if any evidence of illness or infection is detected. If illness is suspected, further advice must be sought from a Designated Frog Recipient (Appendix 2) as soon as possible to determine the nature of the problem. Chytridiomycosis can be diagnosed in live frogs by microscopical examination of preserved toe clips or from shedding skin samples. Research is still in progress on the development of a simple technique for the detection of Chytridiomycosis and a treatment for infected frogs.

Current methods to be used are:

- A technique for the treatment of potentially infected frogs is to place the frogs individually in a 1mg/L benzalkonium chloride solution for 1 hour on days 1, 3, 5, 9, 11 and 13 of the treatment period. Frogs are then isolated/quarantined for two months (G. Marintelli, Amphibian Research Centre pers. comm.) to determine the success of the treatment. This and other possible treatments are documented in Berger and Speare (1998).
- **Betadine**<sup>®</sup> and **Bactonex**<sup>®</sup> treatments have also been used on adult frogs with some success (M. Mahony, Newcastle University pers. comm.).

Frogs undergoing treatment should be housed individually and kept separate from non-infected individuals.

### 3.4 Displaced Frogs

The incidence of frogs being unintentionally transported around the country with fresh produce is common and may significantly impact on local frog species. The introduced Cane Toad (*Bufo marinus*) may also occur in transported produce or landscaping supplies. Procedures to be undertaken when encountering introduced/displaced native frog species (as well as Cane Toads) are as follows.

#### 3.4.1 Banana Box Frogs

'Banana Box' frog is the term used to describe several native frog species (usually *Litoria gracilentia*, *L. infrafrenata*, *L. bicolor* and *L. caerulea*) commonly transported in fruit and vegetable shipments and landscaping supplies. In the past, well meaning individuals have attempted to return these frogs to their place of origin but this is usually impossible to do accurately. There is risk of spread of disease if these frogs are transferred from place to place.

It is strongly recommended that:

***Displaced Banana Box frogs should be treated as if they are infected and are not to be freighted anywhere for release to the wild unless specifically approved by NPWS.***

When encountering a displaced frog:

- Contact a licensed wildlife carer organisation to collect the animal. The frog should then undergo a quarantine period of 2 months along with an approved disinfection treatment.
- Post-quarantine, the frog (if one of the species identified above) may be transferred to a licensed frog keeper. All other species require the permission from NPWS Wildlife Licensing (WLU) prior to transfer. Licensed carer groups are to record and receipt frogs obtained and disposed of in this way.
- Licensed Frog Keepers are to list these frogs in their annual licence returns to NPWS.

Frogs held by licensed Frog Keepers are *not* to be released to the wild except with specific NPWS approval.

Displaced frogs may be made available to recognised institutions for research projects, display purposes or perhaps offered to the Australian Museum as scientific specimens once approval has been provided by the NPWS WLU.

### 3.4.2 Cane Toads

***Cane Toads are known carriers of the Chytrid fungus and should not be knowingly transported or released to the wild.***

If a Cane Toad is discovered outside of its normal range, it should be humanely euthanased in accordance with the recommended NSW Animal Welfare Advisory Council procedure (see Appendix 3). Care should be taken to avoid euthanasia of native species due to mistaken identity. An NPWS information brochure titled '*Cane Toads in NSW*' provides further information on Cane Toads and assistance with identification of some of the commonly misidentified native species.

### 3.4.3 Local Frog Species

***Frogs encountered on roads, around dwellings and gardens or in swimming pools should not be considered as displaced frogs.***

Frogs encountered in these situations should be assisted off roads, away from dwellings, or out of swimming pools preferably to the nearest area of vegetation or suitable habitat.

Incidences of frogs spawning or tadpoles appearing in swimming pools should be referred to a wildlife carer/rescue organisation for assistance (see Appendix 4).

## 4. Sick or Dead Frogs

Unless an obvious cause of illness or death is evident (eg predation or road mortality):

***Sick or dead frogs encountered in the field should be collected and disposed of in accordance with the procedures described in section 4.2 below.***

The NPWS has been involved in a project to determine the areas and species affected by the Chytrid fungus (as well as other diseases) and encourages those finding sick or dead frogs to assist this project.

### 4.1 Symptoms of Sick and Dying Frogs

#### *Appearance (one or more symptoms)*

- darker or blotchy upper (dorsal) surface
- reddish/pink-tinged lower (ventral) surface and/or legs and/or webbing or toes
- swollen hind limbs
- very thin or emaciated
- skin lesions (sores, lumps)
- infected eyes

#### *Behaviour (one or more symptoms)*

- lethargic limb movements, especially hind limbs
- abnormal behaviour (eg a nocturnal, burrowing or arboreal frog sitting in the open during the day and making no vigorous attempt to escape when approached)
- little or no movement when touched

### ***Diagnostic Behaviour Tests***

Sick frogs will fail one or more of the following tests.

<b>Test</b>	<b>Healthy</b>	<b>Sick</b>
Tap with finger above eye	Frog will blink	Frog will not blink
Turn frog on its back	Frog will flip back over	Frog will remain on its back
Hold frog by its mouth	Frog will use its forelimbs to try to remove grip	No response from frog

## **4.2 What To Do With Sick or Dead Frogs**

A procedure for the preparation and transport of a sick or dead frog is given below. Adherence to this procedure will ensure the animal is maintained in a suitable condition for pathological examination and assist the NPWS and researchers to determine the extent of the disease and the number of species affected.

- Disposable gloves should be worn when handling sick or dead frogs. Avoid handling food and touching your mouth or eyes as this could transfer pathogens and toxic skin secretions from some frog species.
- New gloves and a clean plastic bag should be used for each frog specimen to prevent cross-contamination. When gloves are unavailable, use an implement to transfer the frog to a container rather than using bare hands.
- If the frog is dead, keep the specimen cool and preserve as soon as possible (as frogs decompose quickly after death making examination difficult). Specimens can be fixed/preserved in 70% ethanol or 10% buffered formalin.  
Cut open the belly and place the frog in about 10 times its own volume of preservative. Alternatively, specimens can be frozen (although this makes tissues unsuitable for some tests). If numerous frogs are collected, some should be preserved and some should be frozen. Portions of a dead frog can be sent for analysis eg a preserved foot, leg or a portion of abdominal skin.
- The container should be labelled showing at least the species, date and location. A standardised collection form is provided in Appendix 5.
- If the frog is alive but unlikely to survive transportation (death appears imminent), euthanase the frog (see Appendix 3) and place the specimen in a freezer. Once frozen, the specimen is ready for shipment to the address provided below.
- If the frog is alive and likely to survive transportation, place the frog into either a moistened cloth bag with some damp leaf litter or into a plastic bag with damp leaf litter and partially inflated before sealing. Remember to keep all frogs separated during transportation.
- Preserved samples can be sent in jars or wrapped in wet cloth, sealed in bags and placed inside a padded box.
- Send frozen samples in an esky with dry ice (available from BOC/CIG Gas outlets) or with a frozen cooler brick in the bottom of the esky.
- Place live or frozen specimens into a small styrafoam esky (available from K-Mart/Big W for approximately \$2.50).
- Seal esky with packaging tape and address to one of the laboratories listed in Appendix 4.
- Send the package by courier (NPWS use TNT Express Overnight).
- Keep all receipts for esky, ice, cooler bricks and courier for reimbursement from NPWS Threatened Species Unit Northern Directorate (Appendix 3).

Further information on sick and dying frogs is available on the Amphibian Disease Home Page at <http://www.jcu.edu.au/dept/PHTM/frogs/ampidis.htm>

In particular refer to - "*What to do with dead or ill Frogs*"

## 5. References

- Alford, R.A. and Richards, S.J. (1997) Lack of evidence for epidemic disease as an agent in the catastrophic decline of Australian rainforest frogs. *Conserv. Biol.* **11**: 1026-1029.
- Berger, L., Speare, R. (1998) Chytridiomycosis – a new disease of amphibians. *ANZCCART News* **11**(4): 1-3.
- Berger, L., Speare, R., Daszac, P., Green, D.E., Cunningham, A.A., Goggin, C.L., Slocombe, R., Ragan, M.A., Hyatt, A.D., McDonald, K.R., Hines, H.B., Lips, K.R., Marantelli, G. and Parkes, H. (1998) Chytridiomycosis causes amphibian mortality associated with population declines in the rainforests of Australia and Central America. *Proc. Nat. Acad. Sci.* **95**: 9031-9036.
- Berger, L., Speare, R. and Hyatt, A. (1999) Chytrid fungi and amphibian declines: Overview, implications and future directions. *In*: Campbell, A. (Editor) Declines and disappearances of Australian frogs. Biodiversity Group, Environment Australia.
- Ferrero, T.J. and Bergin, S. (1993) Review of environmental factors influencing the declines of Australian frogs. *In*: Lunney, D. and Ayers, D. (Editors) Herpetology in Australia: a diverse discipline. Trans. R. Zool. Soc. Mosman.
- Laurance, W.F., McDonald, K.R. and Speare, R. (1996) Epidemic disease and catastrophic decline of Australian rainforest frogs. *Conserv. Biol.* **77**: 203-212.
- Pechmann, J.H.K. and Wilbur, H.M. (1994) Putting declining amphibian populations into perspective: natural fluctuations and human impacts. *Herpetologica* **50**: 64-84.
- Pechmann, J.H.K., Scott, D.E., Semlitsch, R.D., Caldwell, J.P., Vitt, L.J. and Gibson, J.W. (1991) Declining amphibian populations: the problem of separating human impacts from natural fluctuations. *Science* **253**: 892-895.
- Pounds, J.A. and Crump, M.L. (1994) Amphibian declines and climate disturbance: the case for the golden toad and harlequin frog. *Conserv. Biol.* **8**: 72-85.
- Pounds, J.A., Fogden, M.P.L., Savage, J.M. and Gorman, G.C. (1997) Test of null models for amphibian declines on a tropical mountain. *Conserv. Biol.* **11**: 1307-1322.
- Richards, S.J., McDonald, K.R. and Alford, R.A. (1993) Declines in populations of Australia's endemic tropical rainforest frogs. *Pacific Conserv. Biol.* **1**: 66-77
- Speare, R., Berger, L. and Hines, H. (1999) How to reduce the risk of you transmitting an infectious agent between frogs and between sites. Amphibian Diseases Home Page 22/1/99, (<http://www.jcu.edu.au/dept/PHTM/frogs/ampdis.htm>).

## Appendix 1      Hygiene Protocol Checklist

This checklist is designed to assist with minimising the risk of transferring pathogens between frogs.

*Have you considered the following questions before handling frogs in the field:*

- $\pi$       Has your proposed field trip been sufficiently well planned to consider hygiene issues?
- $\pi$       Have you taken into account boundaries between sites (particularly where endangered species or populations at risk are known to occur)?
- $\pi$       Have footwear disinfection procedures been considered and a strategy adopted?
- $\pi$       Have you planned the equipment you will be using and developed a disinfection strategy?
- $\pi$       Are you are planning to visit sites where vehicle disinfection will be needed (consider both vehicle wheels/tyres and control pedals) and if so, do you have a plan to deal with vehicle disinfection?
- $\pi$       Have handling procedures been planned to minimise the risk of frog to frog pathogen transmission?
- $\pi$       Do you have a planned disinfection procedure to deal with equipment, apparel and direct contact with frogs?

*If you answered **NO** to any of these questions please re-read the relevant section of the NPWS Hygiene Protocol for the Control of Disease in Frogs and apply a suitable strategy.*

## Appendix 2 Designated Sick and Dead Frog Recipients

*Contact one of the following specialists to arrange receipt and analyse sick and dead frogs. Make contact prior to dispatching package*

Lee Berger  
Australian Animal Health Laboratory  
CSIRO Australian Animal Health Laboratory  
Ryrie St.  
**GEELONG VIC 3220**

Contact Lee Berger or Alex Hyatt at the CSIRO AAHL  
[Phone: 03 52275397 or 03 52275000 Fax: 03 52275555 or e-mail [Lee.Berger@dah.csiro.au](mailto:Lee.Berger@dah.csiro.au) ]

Karrie Rose  
Pathology  
Taronga Zoo PO Box 20  
**MOSMAN NSW 2088**  
[Phone: 99784749 Fax: 99784516 [Krose@zoo.nsw.gov.au](mailto:Krose@zoo.nsw.gov.au) ]

Rick Speare (for all skin or toe-clip samples)  
School of Public Health and Tropical Medicine  
James Cook University  
**TOWNSVILLE Qld 4811**  
[Phone: 07 4722 5700 Fax: 07 4722 5788 [Richard.Speare@jcu.edu.au](mailto:Richard.Speare@jcu.edu.au) ]

Michael Mahony  
School of Biological Sciences  
University of Newcastle  
**CALLAGHAN NSW 2308**  
[Phone: 49216014 Fax: 49216923 [bimjm@cc.newcastle.edu.au](mailto:bimjm@cc.newcastle.edu.au) ]

*For reimbursement of costs associated with the transport of sick and dead frogs costs contact:*

Nick Sheppard  
Threatened Species Unit  
NSW National Parks and Wildlife Service (Northern Directorate)  
Locked Bag 914  
**COFFS HARBOUR NSW 2450**  
Phone: 02 6659 8231 Fax: 02 6651 6187  
Email: [nick.sheppard@npws.nsw.gov.au](mailto:nick.sheppard@npws.nsw.gov.au)

*For information on frog keeping licences and approvals to move some species of displaced frog contact:*

The Manager  
NPWS Wildlife Licencing  
Biodiversity Research and Management Division  
Ph 95856481 Fax 95856401  
Email: [jeff.hardy@npws.nsw.gov.au](mailto:jeff.hardy@npws.nsw.gov.au)

## **Appendix 3      NSW    Animal    Welfare    Advisory    Council** **Methodology**

The NSW Animal Welfare Advisory Council Procedure for humanely euthanasing Cane Toads or terminally ill frogs is stated as follows:

- Using gloves, or some other implement, place Cane Toad or terminally ill frog into a plastic bag.
- Cool in the refrigerator to 4°C.
- Crush cranium with a swift blow using a blunt instrument.

*Note:* Before killing any frog presumed to be a Cane Toad, ensure that it has been correctly identified and if outside the normal range for Cane Toads in NSW (north coast) that local NPWS regional office is informed.

## **Appendix 4      Licensed Wildlife Carer and Rescue Organisations**

**Following is a list of wildlife rehabilitation groups licensed by NSW National Parks and Wildlife Service:**

### *Northern NSW*

**A.S.R.** (Australian Seabird Rescue), "Waverly", Pacific Highway, WEST BALLINA 2478  
**FRIENDS OF THE KOALA**, PO.Box 5034, EAST LISMORE 2477  
**K.P.S.N.** (Koala Preservation Society of NSW), PO BOX 236, PORT MACQUARIE 2444  
**N.R.W.C.** (Northern Rivers Wildlife Carers), PO Box 6432, LISMORE SOUTH 2480  
**N.T.W.C.** (Northern Tablelands Wildlife Carers), PO Box 550, ARMIDALE 2350  
**T.V.W.C.** (Tweed Valley Wildlife Carers), PO Box 898, MURWILLUMBAH 2484  
**W.C.G.I.** (Wildlife Carers of Glen Innes), PO Box 520, GLEN INNES 2370

### *Central Coast inc Sydney*

**A.W.A.R.E.** (Aust. Wildlife Ambulance Rescue Service), PO Box 592, CARINGBAH 2229  
**A.W.H.** (Australian Wildlife Hospital), PO Box 84, RAYMOND TERRACE 2324  
**C.C.F.F.C.** (Cabramatta Creek Flying-fox Committee), PO Box 430 BONNYRIGG 2177  
**F.A.W.N.A.** (For Aust Wildlife Needing Aid), P.O. Box 41, BEECHWOOD 2446  
**G.L.W.R.** (Great Lakes Wildlife Rescue), c/- Huntley, The Lakes Way, BUNGWAHL 2423  
**H.K.P.S.** (Hunter Koala Preservation Society), PO Box 544, RAYMOND TERRACE 2324  
**I.F.A.W.** (International Fund for Animal Welfare Aust P/L) 29 Georgina St, NEWTOWN 2042  
**K.B.C.C.** (Ku-ring-gai Bat Colony Committee), 45 Highfield Road, LINDFIELD 2070  
**K.P.C.** (Kangaroo Protection Co-operative), GPO Box 3719, SYDNEY 2001  
**N.A.T.F.** (Native Animal Trust Fund), 8 Conway St, TORONTO 2283  
**O.R.R.C.A.** (Org. for the Rescue and Research of Cetaceans), PO Box 442, ARTARMON 2064  
**S.M.W.S.** (Sydney Metropolitan Wildlife Services), 31 Chiltern Rd, INGLESIDE 2101  
**WILD & FREE**, PO Box 268, GLOUCESTER 2422  
**WILDLIFE ARC** (Wildlife - Animal Rescue & Care), PO Box 2383, GOSFORD 2250  
**WIRES** (Wildlife Information & Rescue Service), PO Box 260, FORESTVILLE 2087  
(Note: WIRES has 25 Branches - addresses available separately)

### *Southern NSW*

**L.A.O.K.O.** (Looking After Our Kosciusko Orphans), 18 Kurrajong St, JINDABYNE 2627  
**N.A.N.A.** (Native Animal Network Assoc.), P.O. Box 780, ULLADULLA 2539  
**S.W.C.G.** (Sunraysia Wildlife Carers Group), PO Box 189, GOL GOL 2738

### *Western NSW*

**L.W.C.** (Lachland Wildlife Carers), 'Revenue', CONDOBOLIN 2877  
**WILDCARE QUEANBEYAN**, PO Box 1404, QUEANBEYAN 2620  
**R.R.A.N.A.** (Rescue & Rehab. of Aust. Native Animals), 107 Boughtman St, BROKEN HILL 2880  
**ACT Wildlife Foundation** - not licensed in NSW. PO Box 207, JAMISON CENTRE ACT 2614  
**WCN(CW)** (Wildlife Carers Network (Central West)), "Grunty Fen", RUNNING STREAM 2850

**Appendix 5**

**Sick or Dead Frog Collection Form**

# SICK OR DEAD FROG COLLECTION FORM

**Sender Details:**

Name: ..... Address: .....

Phone: (W) ..... (H) ..... Fax: ..... E-mail: .....

**Collector Details:** (where different to sender)

Name: ..... Address: .....

Phone: (W) ..... (H) ..... Fax: ..... E-mail: .....

**Specimen Details:**

Record No. : ..... No. of Specimens: ..... Species Name: .....

Date collected(day/mth/yr): . . . / . . . / . . . Time collected: ..... Sex: M/F .....

Status at time of collection (healthy(H)/ sick(S)/ dead(D): ..... Date sent (day/mth/yr): . . . / . . . / . . .

Location: .....

Map Grid Reference: (Easting) ..... (Northing) .....

Reason for collection: .....

**Batch details for multiple species collection:**

Species Name	No.	Locality	(AMG)	Date	Sex	Status

**Habitat Description:**

Habitat Type: (eg Creek, Swamp, Forest) .....

Vegetation Type: (eg rainforest, sedgeland) .....

Micro Habitat: (eg.creek bank, under log, amongst emergent vegetation, on ground in the open) .....

**Unusual behaviour of sick frogs:** eg. lethargic, convulsions, sitting in the open during the day, showing little or no movement when touched. ....

**Dead frogs appearance:** eg. thin, reddening of skin on belly and/or toes, red spots, sore, lumps or discolouration on skin .....

**Deformed frogs:** eg. limb(s) missing, abnormal shape or length. ....

**Dead/Sick Tadpoles:** (eg no.s, behaviour) .....

**Unusual appearance of egg masses:** eg. grey or white eggs .....

**Recent use of agricultural chemicals in the area:** eg pesticides, herbicides, fertilisers .....

**Other potential causes of sickness/mortality/comments/additional information:** .....

## Contact Details

Please send specimens (each with completed copies of this form and the “Sick or Dead Record Form”) to:

Dr. Lee Berger  
CSIRO Animal Health Laboratory  
Ryrie St, GEELONG VIC 3220  
Phone: 03 5227 5209 Fax: 03 5227 5555  
E-mail: [Lee.Berger@dah.csiro.au](mailto:Lee.Berger@dah.csiro.au)

or

Dr. Michael Mahony  
Department of Biological Sciences  
University of Newcastle, CALLAGHAN, NSW 2308  
Phone: 02 4921 6014 Fax: 02 4921 6923  
E-mail: [bimjm@cc.newcastle.edu.au](mailto:bimjm@cc.newcastle.edu.au)

Karrie Rose, Pathology,  
Taronga Zoo PO Box 20  
**MOSMAN NSW 2088**  
*Phone: 99784749 Fax: 99784516*  
[Krose@zoo.nsw.gov.au](mailto:Krose@zoo.nsw.gov.au)

or

Rick Speare, (for all skin or toe-clip samples)  
School of Public Health and Tropical Medicine  
James Cook University,  
**TOWNSVILLE Qld 4811**  
*Phone: 07 4722 5700 Fax: 07 4722 5788*  
[Richard.Speare@jcu.edu.au](mailto:Richard.Speare@jcu.edu.au)

Do **NOT** send specimens before you have contacted the relevant individual above to discuss the most appropriate mode and date of transport.

Currently Northern Directorate TSU is paying packaging and transport costs. Please contact Dr Nick Sheppard to discuss means of reimbursement.

Copies of this form should also be sent to:

Dr. Nick Sheppard  
NSW NPWS, PO Box 914,  
Coffs Harbour 2450.  
Ph: 02 6659 8231 Fax: 02 6651 6187 E-mail: [nick.sheppard@npws.nsw.gov.au](mailto:nick.sheppard@npws.nsw.gov.au)