

Threatened Species Management
Information Circular No. 6



hygiene protocol for the control of disease in frogs

August 2001

NSW
NATIONAL
PARKS AND
WILDLIFE
SERVICE

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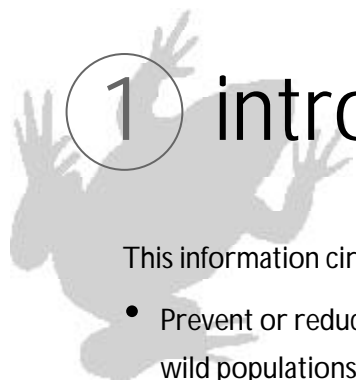
This hygiene protocol is an adaptation of the Declining Amphibian Population Task Force (DAPTF) Fieldwork Code of Practice and the recommendations of Speare et al. (1999) and has drawn on recommendations from earlier guidelines prepared by Environment ACT.

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hygiene protocol for the control of disease in frogs

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A faint, light grey silhouette of a frog is positioned in the upper left corner of the page, behind the main title.

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

1.2.1 Amphibian Chytrid Fungus

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 dendrobatidis* 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 amphibian or frog chytrid fungus and is responsible for the disease Chytridiomycosis (Berger et al., 1999).

B. dendrobatidis is a form of fungus belonging to the phylum Chytridiomycota. Most species within this phylum occur as free-living saprophytic fungi in water and soil and have been found in almost every type of environment including deserts, arctic tundra and rainforest and are considered important primary biodegraders (Powell 1993). *B. dendrobatidis* is a unique parasitic form of Chytridiomycete fungi, in that it invades the skin of amphibians, including tadpoles, often causing sporadic deaths with up to 100% mortality in some populations. Chytridiomycosis has been detected in over 40 species of native amphibian in Australia (Mahony and Wekman 2000). However, it is not currently known whether the fungus is endemic or exotic to Australia.

The infective stage of *B. dendrobatidis* is the zoospore and transmission requires water (Berger et al., 1999). Zoospores released from an infected amphibian can potentially infect other amphibians in the same water. More research is needed on the dynamics of infection in the wild.

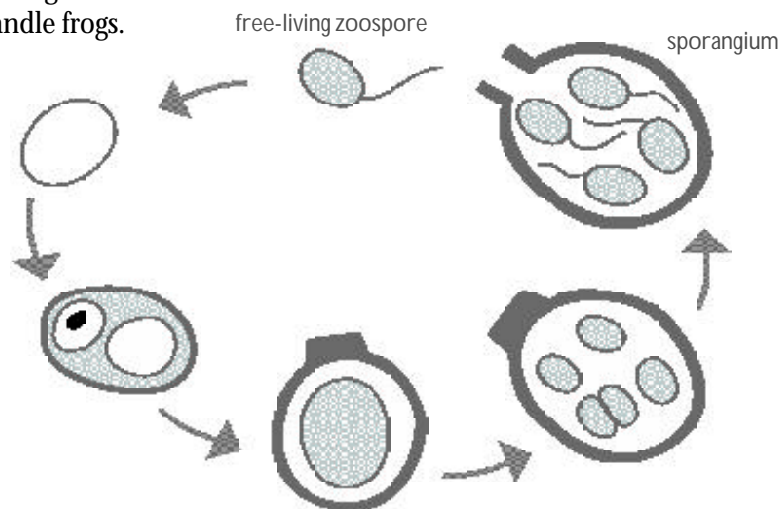
B. dendrobatidis is known to be susceptible to seasonal temperature changes, dehydration, salinity, water pH, light, nutrition and dissolved oxygen (Berger et al., 1999).

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 frog 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.



Life cycle of frog chytrid fungus from infective free-living zoospore stage to sporangium (adapted from L. Berger).

A faint, stylized silhouette of a frog is positioned behind the page number '2' and the title 'site hygiene management'.

2 site hygiene management

A checklist of risk management procedures and recommended standard hygiene kit is provided in Appendix 1. Please note Footnote 1 on page 4.

Individuals studying frogs often travel and collect samples of frogs from multiple sites. Some frog populations can be particularly sensitive to the introduction of infectious pathogens such as the frog chytrid fungus. Also, the arrangement of populations in the landscape may make frogs 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.

When planning to survey multiple sites, always start at a site where frog chytrid fungus is not known to be present before entering other infected areas.

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. Such a case would occur in areas where frogs are known to have free interchange between ponds.

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, particularly if there is no known interchange of frogs between 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 that contains *benzalkonium chloride* as the active ingredient. 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 'toilet duck' (active ingredient *benzalkonium chloride*) is recommended to disinfect car wheels and tyres.

Cleaning of footwear before getting back into the car 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 frog chytrid fungus, may occur as a result of handling frogs.

Frogs should only be handled when necessary.

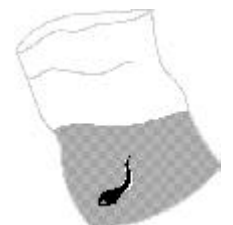
Where handling of frogs is necessary the risk of pathogen transfer should be minimised as follows:

- Hands should be either cleaned and disinfected between samples or a new pair of disposable gloves used for each sample¹. 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.

Researchers who use toe clipping or Passive Integrated Transponder (PIT) tagging are 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

Disinfecting solutions containing *benzalkonium chloride* are readily available from local supermarkets. Some brands include Toilet Duck, Sanpic, New Clenz and Pine Clean.



¹ As a principle, this protocol assumes that not all frogs in an infected pond will be contaminated by the frog chytrid fungus. The infective load of a body of water may not be high enough to cause cross contamination of individual frogs in the same pond. Therefore care should be taken to use separate gloves and bags and clean hands for each sample, to avoid transmission of high infective loads between individuals.

Open wounds from toe clipping and PIT tagging should be sealed with a cyanoacrylate compound such as *Vetbond*® to reduce the likelihood of entry of pathogens. The NPWS ACEC further recommends the application of topical anaesthetic *Xylocaine*® cream and *Betadine*® disinfectant (1% 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 sharps or other waste container and disposed or sterilised 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*®, *Halasept*®

or *Hexifoam*® 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

Fresh bleach (5% concentration) may be also effective against other frog pathogens such as Rana Virus.

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

3

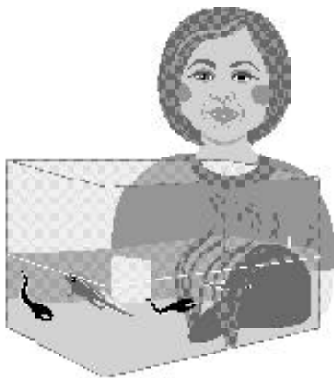
captive frog hygiene management

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.



Careful maintenance of your enclosures will ensure a safe and hygienic environment for captive frogs and tadpoles.

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 will licence the 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 frog chytrid fungus are currently being trialed. In the future, such a treatment may be an added procedure required prior to froglet releases

Detailed information on safely maintaining frogs in captivity is provided in Voigt (2001).

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 which may be used include:

- 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. This and other possible treatments are documented in Berger and Speare (1998)
- *Betadine*® and *Bactone*® treatments have also been used on adult frogs with some success (M. Mahony, Newcastle University pers. comm.)

- *Itraconazole*® is an expensive drug which has been used successfully (Lee Berger CSIRO Australian Animal Health Laboratory pers. comm.). Information on this method is available on the Website <http://www.jcu.edu.au/school/PHTM/frogs/adms/attach6.pdf>.

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

3.4 Displaced frogs

Displaced frogs are those native frog species and introduced Cane Toads (*Bufo marinus*) which have been unintentionally transported around the country with fresh produce, transported produce and 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 should 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.



Frogs are often unintentionally transported with fresh produce and landscaping supplies. They are collectively known as 'banana box' or displaced frogs.

3.4.2 Cane toads

Cane toads are known carriers of the Frog 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.

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).

Contact the Frogwatch Helpline if you are unsure whether a frog is a local species or displaced.

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. This information is also available on the NPWS website.



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 wild should be collected and disposed of in accordance with the procedures described in section 4.2 below.

4.1 Symptoms of sick and dying frogs

Sick and dying frogs exhibit a range of symptoms characteristic of chytrid infection. Symptoms may be expressed in the external appearance or behaviour of the animal. A summary of these symptoms are described below. More detailed information can be found in Berger et al., (1999) or at the James Cook University Amphibian Disease website at: <http://www/jcu.edu.au/school/phtm/PHTM/frogs/ampdis.htm>. A directory of other useful websites is provided in NPWS (2001).



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
- obvious asymmetric appearance

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

Great barred frog (*Mixophyes fasciolatus*) with severe Chytrid infection — note lethargic attitude and sloughing skin. Photo: L. Berger

Diagnostic behaviour tests

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

test	healthy	sick
Gently touch with finger	Frog will blink	Frog will not blink above the eye
Turn frog on its back	Frog will flip back over	Frog will remain on its back
Hold frog gently 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 below². 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).
- 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'.

² The measures described below are standard procedures and may vary slightly depending on the distance and time required to reach the intended recipient. Contact the intended recipient of the sick or dead frog prior to sending to confirm the appropriate procedure.



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.

Environment ACT (1999) Guidelines for minimising introduction and spread of frog pathogens. Environment ACT. Canberra.

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.

Mahony, M. and Werkman, H. (2000) The distribution and prevalence of Chytrid fungus in frog populations in eastern New South Wales and developing a means to identify presence or absence of Chytrid fungus in the field. Unpublished report to NSW National Parks and Wildlife Service.

National Parks and Wildlife Service (2000) *Helping frogs survive- A guide for frog enthusiasts*. (Prepared by Voight, L., Haering, R., and Wellington, R). NPWS Hurstville. NSW.

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.

Powell, M.J. (1993) Looking at mycology with a Janus face: A glimpse of chytridiomycetes active in the environment. *Mycologia* 85: 1-20.

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>).

Voight, L. (2001) Frogfacts No. 8. Frog hygiene for captive frogs (draft publication). FATS. Group. Sydney.

appendix 1

hygiene protocol checklist and field kit

The following checklist and field kit are designed to assist with minimising the risk of transferring pathogens between frogs.

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

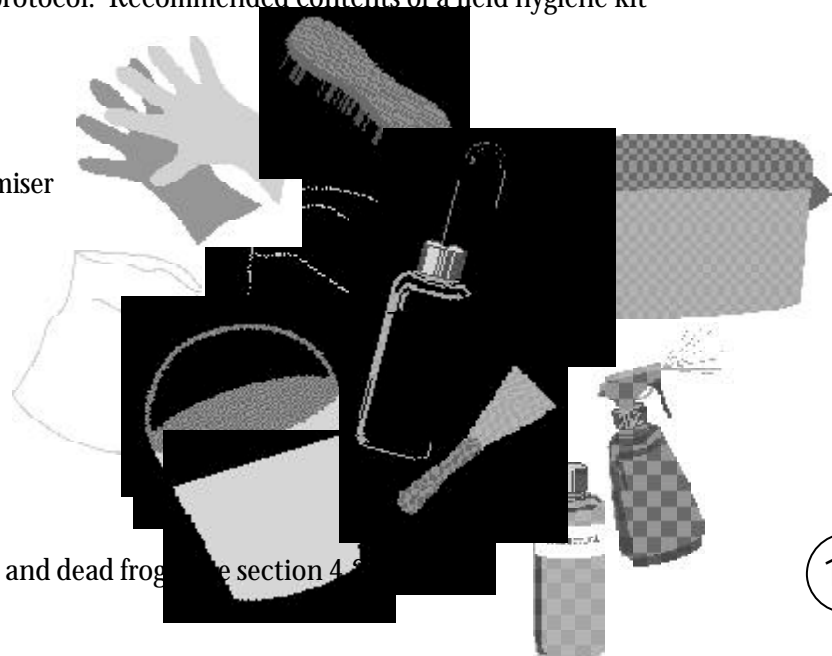
- Has your proposed field trip been sufficiently well planned to consider hygiene issues?
- Have you taken into account boundaries between sites (particularly where endangered species or populations at risk are known to occur)?
- Have footwear disinfection procedures been considered and a strategy adopted?
- Have you planned the equipment you will be using and developed a disinfection strategy?
- 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?
- Have handling procedures been planned to minimise the risk of frog to frog pathogen transmission?
- 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.

Field hygiene kit

When planning to survey frogs in the field a portable field hygiene kit should be assembled to assist with implementing this protocol. Recommended contents of a field hygiene kit would include:

- Small styrofoam eski
- Disposable gloves
- Disinfectant spray bottle (atomiser spray) and/or wash bottle
- Disinfecting solutions
- Wash bottle
- Scraper or scrubbing brush
- Small bucket
- Plastic bags large and small
- Container for waste disposal
- Materials for dealing with sick and dead frogs (see section 4)



appendix 2

Always contact the relevant specialist prior to sending a sick or dead frog. In some cases, only wild frogs will be assessed for disease. Analysis may also attract a small fee per sample.

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:

Karrie Rose
Pathology Taronga Zoo
PO Box 20
MOSMAN NSW 2088

Phone: 02 9978 4749
Fax: 02 9978 4516
Krose@zoo.nsw.gov.au

Diana Mendez
School of Public Health
and Tropical Medicine
JCU House — Suite 11
57 Mitchell Street
North Ward
TOWNSVILLE QLD 4810

Phone: 07 4722 5771 or 07 4781 4181
Fax: 07 4722 5788
Diana.Mendez@jcu.edu.au

for all skin or toe-clip samples:

Rick Speare
School of Public Health
and Tropical Medicine
JCU House — Suite 13
57 Mitchell Street
North Ward
TOWNSVILLE QLD 4810

Phone: 07 4722 5777
Fax: 07 4722 5788
Richard.Speare@jcu.edu.au

Michael Mahony
School of Biological Sciences
University of Newcastle
CALLAGHAN NSW 2308

Phone: 02 4921 6014
Fax: 02 4921 6923
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
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 Licensing
Biodiversity Research and
Management Division
PO Box 1967
Hurstville NSW 2220

Ph 02 9585 6481
Fax 02 9585 6401
jeff.hardy@npws.nsw.gov.au

For information on the possible identity of displaced frogs contact:

Frog and Tadpole Society (FATS)
Frogwatch Helpline

Ph: 0419 249 728

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 Service⁴:

Northern NSW

ASR (Australian Seabird Rescue), 'Waverly',
Pacific Highway, WEST BALLINA 2478

FRIENDS OF THE KOALA,
PO Box 5034, EAST LISMORE 2477

KPSN (Koala Preservation Society of NSW),
PO BOX 236, PORT MACQUARIE 2444

NRWC (Northern Rivers Wildlife Carers),
PO Box 6432, LISMORE SOUTH 2480

NTWC (Northern Tablelands Wildlife
Carers), PO Box 550, ARMIDALE 2350

TVWC (Tweed Valley Wildlife Carers),
PO Box 898, MURWILLUMBAH 2484

WCGI (Wildlife Carers of Glen Innes),
PO Box 520, GLEN INNES 2370

Central Coast including Sydney

AWARE (Aust. Wildlife Ambulance Rescue
Service), PO Box 592, CARINGBAH 2229

AWH (Australian Wildlife Hospital),
PO Box 84, RAYMOND TERRACE 2324

CCFFC (Cabramatta Creek Flying-fox
Committee), PO Box 430 BONNYRIGG 2177

FAWNA (For Aust Wildlife Needing Aid),
PO Box 41, BEECHWOOD 2446

GLWR (Great Lakes Wildlife Rescue), c/-
Huntley, The Lakes Way, BUNGWAHL 2423

HKPS (Hunter Koala Preservation Society),
PO Box 544, RAYMOND TERRACE 2324

IFAW (International Fund for Animal Welfare
Aust P/L), 29 Georgina St, NEWTOWN 2042

KBCC (Ku-ring-gai Bat Colony Committee),
45 Highfield Road, LINDFIELD 2070

KPC (Kangaroo Protection Co-operative),
GPO Box 3719, SYDNEY 2001

NATF (Native Animal Trust Fund),
8 Conway St, TORONTO 2283

ORRCA (Org. for the Rescue and Research of
Cetaceans), PO Box 442, ARTARMON 2064

SMWS (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

LAOKO (Looking After Our Kosciusko
Orphans), 18 Kurrajong St, JINDABYNE 2627

NANA (Native Animal Network Assoc.),
P.O. Box 780, ULLADULLA 2539

SWCG (Sunraysia Wildlife Carers Group),
PO Box 189, GOL GOL 2738

Western NSW

LWC (Lachland Wildlife Carers),
'Revenue', CONDOBOLIN 2877

WILDCARE QUEANBEYAN,
PO Box 1404, QUEANBEYAN 2620

RRANA (Rescue & Rehabilitation of
Australian Native Animals),
107 Boughtman St, BROKEN HILL 2880

RSPCA (ACT) Inc.
PO Box 3082
WESTON ACT 2611

WCN(CW) (Wildlife Carers Network
(Central West)), 'Grunt Fen',
RUNNING STREAM, NSW 2850

⁴ Note: some of these organisations may not care for frogs.

appendix 5 — sick or dead frog collection form

Sender details:

name: _____ address: _____ postcode: _____
phone: (w) _____ (h) _____ fax: _____ email: _____

Collector details: (where different to sender)

name: _____ address: _____ postcode: _____
phone: (w) _____ (h) _____ fax: _____ email: _____

Specimen details:

record no: _____ no. of specimens: _____ species name: _____ date collected: _____
day/month/year
time collected: _____ sex: _____ status at time of collection: _____ date sent: _____
male/female healthy(H)/ sick(S)/ dead(D) day/month/year
location: _____ map grid reference: _____
(easting) (northing)
reason for collection: _____

Batch details for multiple species collection:

species	no.	locality	(AMG)	date	sex	status (H/S/D)

habitat type: _____ vegetation type: _____ micro habitat: _____
eg creek, swamp, forest eg rainforest, sedgeland 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: _____ dead/sick tadpoles: _____
eg limb(s) missing, abnormal shape or length eg numbers/behaviour

unusual appearance of egg masses: _____ recent use of agricultural chemicals in area: _____
eg grey or white eggs eg pesticides, herbicides, fertilisers

other potential causes of sickness/mortality/comments/additional information:



NSW
NATIONAL
PARKS AND
WILDLIFE
SERVICE

General inquiries: PO Box 1967 Hurstville NSW 2220

Phone: 1300 36 1967

Fax: 02 9585 6555 Web site: www.npws.nsw.gov.au

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