

Technical Manual

Wildlife management

Interim hygiene protocol for handling amphibians

This technical manual aims to outline standard measures to be followed to prevent or reduce the spread of disease causing pathogens being transferred within and between frog populations. These hygiene protocols can be applied in both a scientific and general public capacity.

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Purpose

The purpose of this document is to provide standard operating procedures for hygiene protocols when handling amphibians to reduce the risk of disease and parasite transmission between populations during authorised activities. It is recognised that there is a 'natural' or background level of disease and parasite transmission within and between amphibian populations. Applicants and assessors need to evaluate the proposed activities to determine the relative risk of disease transmission, above background levels, and implement appropriate strategies to minimise this risk.

Background

The past 30 years has seen alarming declines in amphibian populations and the extinction of numerous amphibian species the world over. Many of these declines and extinctions appear attributable to recently-emerged infectious diseases, in particular chytridiomycosis and ranaviral disease. While the subject of conjecture, the emergence of these diseases is most likely attributable to the spread of novel (i.e. exotic) pathogens to new areas where amphibians have little or no immunity to infection. Human activities that target amphibians or their habitats have the potential to spread disease and parasites both within and amongst amphibian populations.

Queensland's native wildlife is protected under the *Nature Conservation Act 1992*. Under the Act persons are required to hold licences, permits or authorities to take, use or interfere with native amphibians in many situations. The *Nature Conservation (Wildlife Management) Regulation 2006* outlines these provisions. This policy relates to the following licence and permit types under the regulation:

- Commercial wildlife licence (wildlife interaction)
- Commercial wildlife harvesting licences
- Educational purposes permit
- Museum licence
- Recreational wildlife harvesting licences
- Rehabilitation permit (endorsed for spotter catcher activities)
- Scientific purposes permit

In the course of undertaking activities with native amphibians under these permits or licences the licensee has the potential to increase risk of disease and parasite transmission within and between wild populations and thus threaten the conservation of wild populations.

The *Nature Conservation (Wildlife Management) Regulation 2006* ensures that the granting of such licences does not adversely affect the conservation of the animals and allows the chief executive to limit and regulate the activities of the licensee.

Site Hygiene Management

Frog populations can be very sensitive to any introduced foreign pathogens, so every caution should be taken to minimise the potential risk of contamination transferred between sites. It is for this reason that defining the boundaries between each site, while difficult, is an important recognition to make. Given the possible locations of frog populations, boundaries will be different within each site, some more obvious than others. These boundaries may be natural, such as a body of water, or constructed, such as a road or track. As a guiding principle, each individual water body should be defined as a separate site. When working along the same water body, or series of connected water bodies, this can usually be treated as one site. However, if the water body is

fed through several catchments or tributaries, or if there is a clear break or partition, they should be treated as separate sites.

Above the natural and background transmission rate of disease spread, factors likely to increase the risk of infection include:

- the remoteness of the chosen site
- activities prior to entering the site
- activities being undertaken in the site
- status of disease in the site and surrounding area

Requirements for managing disease in the field and between sites

There is no scientific evidence that the amphibian chytrid fungus or other pathogens of amphibians have been transmitted between water catchments by vehicles, footwear or clothing. There is an absence of hard data on amphibian disease epidemiology and transmission. As a result of this 'data deficiency' a precautionary approach has been taken in developing requirements to manage disease in the field. It is assumed that some human activities may unintentionally increase the risk of spreading diseases between sites.

Hygiene and disease are controlled to a large extent by proper cleaning, disinfecting and/or sterilising:

Cleaning involves the physical removal of organic and inorganic matter from items. Cleaning will not remove pathogens from the items, but it is a necessary step that allows the disinfecting agent to come into direct contact with pathogens on the actual surface of the item. Disinfecting an item by washing it with a proper chemical agent (see Table 1) will reduce the bacteria load to a point where they will not serve as a source of infection, but will continue to persist at low levels on the item.

Sterilisation through the use of heat, chemicals or radiation will remove all life from the item. Disinfection of items should always be done at a safe distance from bodies of water so that the solution infiltrates the soil rather than runs directly into the water. When travelling from site to site, the following hygiene precautions must be taken to minimise the transfer of disease.

Gloves

Delicate species such as frogs may be placed at greater risk when gloves are used because of a loss of tactile sensitivity by the handler. If frogs are handled using bare hands it is extremely important to ensure that the handler has not applied insect repellents, perfumes, lotions, or other potentially toxic substances that might be absorbed through highly permeable amphibian skin.

There is evidence that wearing disposable gloves when handling amphibians will protect the animals' skin from abrasion, chemicals and the spread of infection. However, gloves containing talc should not be worn as they could irritate the amphibian's skin. Gloves should be non-powdered and talc-free or rinsed in warm water prior to use. Vinyl and nitrile gloves are preferred, as latex has been shown to have toxicity towards frog embryos and tadpoles. When handling highly toxic amphibians, gloves should always be worn to protect the handler and contact with bare skin or mucus membranes avoided.

Footwear

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

This is done by:

- Scraping boots clear of mud and standing the soles in an approved disinfecting solution (refer Table 1).

- The remainder of the boot should be rinsed or sprayed with an approved disinfecting solution (refer Table 1).
 - 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 is an alternative to cleaning.

Equipment

Care must be taken to avoid removal of the protective mucus layer covering the skin of amphibians. If nets are used, they should be made of soft cloth materials. Animals should be handled gently and transported in containers that protect them from trauma and injury.

- Equipment such as nets, balances, callipers, bags, scalpels, headlamps, torches, wetsuits and waders that are used at one site must be cleaned and disinfected before re-use at another site. Refer to Table 1 for recommended disinfectants and procedures.
- If equipment is to be used immediately at another site it should be dried out completely before reuse. This can be achieved by having two sets of gear, one of which is drying while the other is in use. Nets and other equipment should be thoroughly rinsed off as even trace amounts of residual bleach can adversely affect aquatic life on contact.
- 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.
- In the field, bleach solutions should be disposed of far from any water body by pouring over a hard surface (such as concrete or bitumen) where it can break down in sunlight and evaporate. If no hard substrates are available, it can be disposed of into the soil away from water bodies.
- For future consideration in a laboratory or research situation, there is an automatic waste-water disinfection system available for complete and proper disposal of chlorinated water.

Vehicles

- Transmission of disease from vehicles is unlikely. However, if a vehicle is used to travel through 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.
- Vehicle cleaning should be carried out at a distance from water bodies that allows the disinfecting solution to infiltrate soil rather than run-off into a nearby water body.
- Vehicle tyres should be sprayed/flushed with a disinfecting solution (refer Table 1) in high-risk areas.
- Ideally, if location and time permits, vehicles should be washed at a carwash between sites.

Handling of amphibians in the wild

The spread of disease, such as the chytrid fungus, may occur as a result of handling frogs. Unnecessary handling should be avoided and the specimen released as soon as possible. The duration of handling should be as short as possible as handling procedures that are quick, even if they are potentially painful, may have less affect on stress levels than longer, less invasive procedures.

Amphibian handling guidelines:

Passive Integrated Transponder (PIT):

- Toe clipping or 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.
- When handling amphibians, the handler should wear unused disposable gloves (refer to section on Gloves p.3) or capture and handle frogs in single use lightweight plastic bags.
- Bare hands may be used provided they are wiped before each capture with a sterilising alcohol-based hand disinfectant.

This can be minimised by:

- using disposable sterile instruments;
 - using instruments disinfected previously and used once;
 - using instruments disinfected in between each frog (refer Table 1);
 - sealing open wounds from toe clipping and PIT tagging with a cyanoacrylate compound such as *Vetbond™* to reduce the likelihood of entry of pathogens;
 - applying topical anaesthetic *Xylocaine™* cream and an iodine-based disinfectant solution before and after any surgical procedure is recommended. This should then be followed by the wound sealant;
 - storing all used disinfecting solutions, gloves and other disposable items 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.

Recommended disinfection strategies (refer Table 1) are available for a range of purposes to reduce risks associated with the amphibian chytrid fungus and ranaviruses.

Swabbing protocol:

Frogs are susceptible to handling stress, which is most likely due to changes in their body temperature. The following techniques are recommended to be followed when swabbing:

- Capture the frog by hand using sterile gloves, clean hands or clean plastic (freezer) bags, with minimal handling time (no more than 30 seconds).
- Transfer the frog into a clean, small plastic clip-seal bag, insert the swab, and close the seal as far as possible. At all times, handle the bag by the corners to minimise heat-transfer from the captor's hands to the frog.
- As the frogs will typically climb upwards, invert the bag.
- Coax the frog into one of the top corners using the swab.
- Swab the frog by rubbing firmly with the swab the body areas to be sampled (e.g. venter, underside of legs, hands and feet).

If, at any time, the frog shows signs of undue stress, such as loss or reduction of limb function, seal the bag in which it is housed and cease handling immediately. Keep the animal under observation in a safe place until it recovers. Once the animal appears healthy, release it without further handling.

Disinfecting procedures

Equipment such as wetsuits, waders, footwear, nets, buckets, vehicles and surgical equipment may act as carriers of disease, particularly if used in multiple sites.

Household bleach (refer Table 1) is recommended for disinfection as it is widely available and is easy to dispose of under field conditions if proper precautions are followed (refer p4). There are other disinfectants as outlined in Table 1 that have been tested and shown to kill target pathogens. It is important to ensure that disinfectants do not leave any residue on equipment, as the residue can be harmful to frogs and their environment.

Table 1: Disinfecting procedures

Application	Disinfectant	Concentration	Time	Method Target	Pathogen
Surgical equipment	Benzalkonium chloride	2mg/ml	1 min	Immerse	<i>B.dendrobatidis</i>
	Ethanol	70%	1 min	Immerse	<i>B.dendrobatidis</i> Ranaviruses
Collection containers, equipment, vehicles	Sodium hypochlorite* (bleach)	4%	15 min	Immerse	Ranaviruses
	Sodium hypochlorite* (bleach)	1%	1 min	Immerse	<i>B. dendrobatidis</i>
	Didecyl dimethyl ammonium chloride	1 in 500 dilution	0.5 min	Immerse	<i>B. dendrobatidis</i>
	TriGene™	1 in 5000 dilution	1 min	Immerse	<i>B. dendrobatidis</i>
	F10™ #	1 in 1500 dilution	1 min	Immerse	<i>B. dendrobatidis</i>
	Virkon™	2 mg/ml	1 min	Immerse	<i>B. dendrobatidis</i> Ranaviruses
	Complete drying		>3 hr		<i>B. dendrobatidis</i>
	Heat	60°C	30 min		<i>B. dendrobatidis</i> Ranaviruses
	Heat	37°C	8 hr		<i>B. dendrobatidis</i>
	Sterilising UV light		1 min		Ranaviruses only
Footwear	Sodium hypochlorite* (bleach)	1%	1 min	Scrub/spray footwear	<i>B. dendrobatidis</i>
	Sodium hypochlorite* (bleach)	4%	15 min	Immerse footwear	Ranaviruses
	Didecyl dimethyl ammonium chloride	1 in 500 dilution	0.5 min	Scrub/spray footwear	<i>B. dendrobatidis</i>
	TriGene™	1 in 5000 dilution	1 min	Scrub footwear	<i>B. dendrobatidis</i>
	F10™ #	1 in 1500 dilution	1 min	Scrub footwear	<i>B. dendrobatidis</i>
	Complete drying		>3 hr		<i>B. dendrobatidis</i>
Cloth (e.g. carry bags)	Hot wash	60°C or greater	30 min		<i>B. dendrobatidis</i> Ranaviruses

*See Equipment section (p4) for recommended in-field disposal methods for bleach.

F10 has been used to treat fungal infections in reptiles and birds, but there does not appear to be data on its safety for amphibians. Caution is recommended when using this product.

Handling of captive amphibians

The greatest risk of transmission of infectious agents is when amphibians are placed

- 1) together in contact,
- 2) in the same container or
- 3) in reused containers without disinfection between animals.

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

- Animals obtained at different sites are to 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 associated equipment) must be sterilised, disinfected and dried immediately after frogs/tadpoles are removed; and
- All equipment must be thoroughly sterilised (or discarded if disposable) between animals when dealing with animals from different enclosures.
- If there is no water available for washing hands between amphibians, the handler should wear unused disposable gloves, or wear an unused plastic bag, or wipe their hands with a sterilising alcohol-based hand disinfectant between amphibians. Ideally, new gloves should be worn for each specimen, as disinfecting will not harm the pathogens DNA, producing potentially false-positive results if testing for disease.
- No more than one terrestrial individual should be held in the same container simultaneously.
- Tadpoles for release should not be held with batches of tadpoles collected from other sites in the same or different water bodies.
- Dead amphibians or amphibians that are obviously ill should be regarded as a higher infection risk than clinically normal amphibians and should be handled with gloves or plastic bags.

Basic guidelines for captive amphibians

The risk levels vary according to the conditions in which the frogs are kept. Some examples are:

- Low risk level: single indoors frog cage.
- Higher risk: frog cages in the house, frog pond outside.
- Very high risk: frog cages indoors, frog pond outside; also trading/swapping/conducting frog rescue/quarantine.

Whichever scenario applies, some basic principles to follow are:

- Keep your frogs at a continually reasonable level of quarantine by having a two-way isolation between cages, and between them and your garden.
- Have an intensive level of quarantine for new frogs and for cages in which a frog has become ill or died.
- Service the least likely to be infected cages first, as spreading even a droplet of water from an infected cage to a healthy one is all that it takes for disease transmission.
- Limit the number of frogs you collect and keep your hands out of their cages as much as possible. Look for screw-on or plug-in food jars which can be changed outside of the cage.
- Keep ants out of the frog enclosures as they have the potential to transport pathogens.
- Do not empty waste water from the enclosures into stormwater drains or onto your garden. Pour into the toilet and wrap solid waste and put into the bin.
- Maintain your frogs' health and resistance to disease by looking after them properly.

Sick or dead frogs

Frogs may be diagnosed by using some basic identifiers in the following table:

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

Sick or dead frogs found in the wild should be collected and disposed of using the following procedures described below:

- Use disposable gloves or plastic bags and avoid handling food or touching your mouth or eyes.
- New gloves or plastic bags should be used for each frog specimen.
- If the frog is dead, keep the specimen cool and preserve as soon as possible to slow decomposition
- If the frog is alive but unlikely to survive transportation, euthanize the frog and place in a freezer. Once frozen, it is ready for transportation to the appropriate officials for diagnosis.
- If the frog is alive and likely to survive transportation, place it in a moistened cloth bag with some damp leaf litter and transport to the appropriate officials for diagnosis.

When in doubt, the precautionary principle should be used, along with the highest standard of cleanliness possible. Additional time spent ensuring proper disinfecting of equipment is always recommended.

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