HYGIENE PROTOCOL FOR HANDLING AMPHIBIANS IN FIELD STUDIES

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General principles and background

1. Effective wildlife management is based on sound scientific evidence and collection of this evidence sometimes requires handling, measurement and manipulation of wild amphibians.
2. Hygiene protocols should be guided by the best available scientific evidence.
3. Hygiene protocols must be practical to carry out under field conditions.
4. Wild amphibians are naturally at risk of exposure to infectious disease via contact with the environment such as water and moist substrates and other amphibians. The number and level of pathogens encountered through these pathways represent the background risk of transmission of pathogens to amphibians.
5. The most severe diseases of wild amphibians are chytridiomycosis, caused by the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*), and to a lesser extent, ranaviral disease caused by Ranaviruses. Outbreaks of chytridiomycosis have been documented in wild amphibians in eastern Australia, environs of Adelaide and southwest Western Australia. No outbreaks of ranaviral disease in wild amphibians have been detected in Australia although one ranavirus, the Bohle iridiovirus, occurs in the wild in Australia.
6. Once the amphibian chytrid fungus is present in a water body it appears to naturally spread throughout that water body.
7. Handling of amphibians should be done in a manner that does not significantly increase their risks of exposure to infectious disease above those normally experienced in the absence of handling. People handling amphibians should not be expected to reduce risks below the natural level for those amphibians.
8. Current data do not indicate that scientific activities have played a significant role in the transmission of chytridiomycosis or other pathogens of amphibians in the wild in Australia or any other country.
9. There is no evidence that the amphibian chytrid fungus or other pathogens of amphibians have been transmitted between water catchments by vehicles, footwear or clothing
10. As the amphibian chytrid fungus is extremely sensitive to temperatures above 29°C and will die at 32°C, B. *dendrobatidis* will not grow on human skin.

Ranaviruses, the other major pathogen of amphibians, also show a sensitivity to temperature, being unable to grow above 33°C.

11. Complete drying will kill the amphibian chytrid fungus, but will not kill ranaviruses.
12. The greatest risk of transmission of infectious agents is when amphibians are placed together in contact or in the same container or in containers reused for holding amphibians without disinfection between amphibians.
13. Effective disinfection strategies (See Table 1), based on scientific evidence, are available for a range of purposes to reduce risks associated with the amphibian chytrid fungus and with ranaviruses.
14. Amphibians have a range of powerful natural anti-microbial agents in their skin which may be responsible for the low incidence of infection after toe tip clipping.
15. Amphibians do not appear to show signs of stress after handling; however, unnecessary handling should be avoided.
16. 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 procedures.

Specific actions

1. Amphibians can be handled using bare hands as long as the handler washes their hands between amphibians in water to which the animals would normally be exposed; this will ensure that the risks to frogs of exposure are not increased above environmental levels.
2. If no water is 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.
3. If amphibians are held in a container prior to return to the wild, the container should not have previously have been used for holding other amphibians, or if previously used, the container should be disinfected prior to use using methods given in Table 1.
4. Surgical instruments, such as scissors used for toe tip clipping, should be sterilised between amphibians by chemical disinfection using 70% ethanol or other chemicals listed in Table 1.
5. When toe tip clipping is used, no more than 50% of the free length of the digit should be removed.
6. Amphibians should be handled and released as quickly as possible.
7. Amphibians should be released at the site from which they were captured.
8. No more than one terrestrial individual should ever be held in the same container simultaneously.
9. Tadpoles normally share water and placing them in a common container does not increase their rates of physical contact. They can therefore be held in groups in containers, as long as all members of the group are from the same site.
10. Tadpoles for release should not be held with batches of tadpoles collected from other sites in the same or different water bodies.

11. Non-surgical equipment used in a stream or water body should be disinfected using one of the methods listed in Table 1 prior to use in any other water bodies.
12. Footwear should be washed to remove any mud and disinfected using one of the methods listed in Table 1 prior to being used in a separate water catchment or water body isolated from the initial water body.
13. As there is no evidence that vehicles play a role in dissemination of the amphibian chytrid fungus, no action is required at this time.
14. 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. If a sick or freshly dead wild amphibian is found, it should be collected, preserved and submitted for disease diagnosis.

Literature Cited

Berger L. Diseases in Australian Frogs [PhD thesis]. Townsville, Australia. James Cook University: Townsville. 2001.

Johnson M, Berger L, Philips L, Speare R**.** Fungicidal effects of chemical disinfectants, UV light, dessication and heat on the amphibian chytrid, *Batrachochytrium dendrobatidis.* Diseases of Aquatic Organisms 2003;57:255-260.

Langdon JS. Experimental transmission and pathogenicity of epizootic haematopoietic necrosis virus (EHNV) in red fin perch, *Perca fluviatilis* L., and 11 other teleosts. Journal of Fish Diseases 1989;12:295-310.

Miocevic I, Smith J, Owens L, Speare R. Ultraviolet sterilisation of model viruses important to finfish aquaculture in Australia. Australian Veterinary Journal 1993;70:25-27.

Table 1: Disinfection strategies suitable for killing *Batrachochytrium dendrobatidis* and ranaviruses in field studies. Where concentrations and time are given, these are minimum shown to be effective. For *B. dendrobatidis* based on Berger (2001) and Johnson et al (2003) and for ranaviruses on Langdon (1989) and Miocevic et al (1993).

Purpose	Disinfectant	Concentration	Time	Pathogen killed
Disinfecting surgical equipment and other instruments (eg, scales)	Ethanol	70%	1 min	*B. dendrobatidis* Ranaviruses
	Vircon	1 mg/ml	1 min	*B. dendrobatidis* Ranaviruses
	Benzalkonium chloride	1 mg/ml	1 min	*B. dendrobatidis*
Disinfecting collection equipment and containers	Sodium hypochlorite (bleach)	1%	1 min	*B. dendrobatidis*
	Sodium hypochlorite (bleach)	4%	15 min	Ranaviruses
	Didecyl dimethyl ammonium chloride	1 in 1000 dilution	0.5 min	*B. dendrobatidis*
	Complete drying		3 hrs or greater	*B. dendrobatidis*
	Heat	60°C	5 min	*B. dendrobatidis*
			15 min	Ranaviruses
	Heat	37°C	4 hrs	*B. dendrobatidis*
	Sterilising UV light		1 min	Ranaviruses only
Disinfecting footwear	Sodium hypochlorite (bleach)	1%	1 min	*B. dendrobatidis*
	Sodium hypochlorite (bleach)	4%	15 min	Ranaviruses
	Didecyl dimethyl ammonium chloride	1 in 1000 dilution	1 min	*B. dendrobatidis*
	Complete drying		3 hrs or greater	*B. dendrobatidis*
Disinfecting cloth (eg, bags, clothes)	Hot wash	60°C or greater	5 min	*B. dendrobatidis*
			15 min	Ranaviruses