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Guidelines for the Safe Use of Disposable Gloves with Amphibian Larvae in Light of Pathogens and Possible Toxic Effects

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Prior to the discovery and widespread recognition that pathogens such as *Batrachochytrium dendrobatidis* (*Bd*) and ranaviruses can lead to the decline and even extinction of amphibian populations around the world, few herpetologists used gloves when handling amphibians. Disposable gloves, along with disinfection protocols (Webb et al. 2007), quarantine (Young et al. 2007), and reports by Daszak et al. (2001) and Lynch (2001) have since become an important tool in our arsenal for preventing the spread of pathogens between individuals and among populations or habitats.

Cashins et al. (2008) recently reported that latex, vinyl, and nitrile gloves can be lethal to tadpoles of several species (*Litoria genimaculata*, *L. nannotis*, and *Bufo marinus*), even after short-term exposures during routine handling. Two previous studies had also found that latex and nitrile gloves (or water in which gloves had been soaked) could be lethal to *Xenopus laevis* and *Rana temporaria* tadpoles (Sobotka and Rahwan 1994, Gutleb et al. 2001). In an unrelated study aimed at developing hygiene protocols to prevent the spread of *Bd* among animals, Mendez et al. (2008) reported that bare hands that are washed between animals may be

Table 1. Glove related contact and associated morbidity and mortality for larval amphibians involved in laboratory experiments. All experimental animals were examined for signs of morbidity or mortality daily.

Species	Origin	N	Frequency of contact	Contact type[1]	Contact duration	Type of gloves used[2]	Response[3]	Reference
Wood Frog (*Rana sylvatica*)	Ontario, Canada	240	weekly for 6 weeks	SVL STAGE HUS	2–3 min	P-LTX	None observed	Greer et al. (2005)
Arizona Tiger Salamander (*Ambystoma tigrinum nebulosum*)	Lab bred, Arizona, USA, stock	1038	weekly for 4 weeks	VIE CLIP HUS	45 s	P-LTX N-LTX	None observed	Greer et al. (2008)
	Arizona, USA	335	weekly for 8 weeks	HUS	30 s	P-LTX	None observed	Brunner et al. (2005)
Gray Tiger Salamander (*Ambystoma tigrinum diaboli*)	Saskatchewan and Manitoba, Canada	397	weekly for 16–20 weeks	HUS SVL STAGE	1 min	P-LTX	None observed	D. Schock, pers. comm.

[1] SVL = snout–vent length measurement, STAGE = developmental stage, CLIP = tissues sample, SWAB = swab for DNA, VIE = visible implant elastomer tag marking, HUS = animal husbandry/water changes
[2] P-LTX = powdered latex, N-LTX = non-powdered latex, P-NIT = powdered nitrile
[3] Based on description of morbidity and mortality described by Cashins et al. (2008)

preferable to repeatedly using the same pair of gloves in some situations. Although both Mendez et al. (2008) and Cashins et al. (2008) conclude that using new pairs of gloves for each handled animal is important for preventing the inadvertent transmission of pathogens, we have become aware that some organizations and researchers are interpreting these studies as cause, or even justification, for not using gloves. We wish to emphasize that discontinuing glove use may unnecessarily lead to increased spread of harmful pathogens.

Although these studies indicate limitations and potential pitfalls of disposable gloves, their safe use remains an essential component of amphibian care and research. A variety of disposable gloves have been used extensively in the handling and care of larval amphibians in laboratory experiments (Table 1), in the field (Table 2), and in zoo settings (Table 3) without any adverse effects under conditions where morbidity or mortality would have been noticed. Additional examples documenting the non-injurious use of gloves appear in Cashins et al. (2008). These results make it clear that many glove types do not negatively affect many species of amphibian larvae. Given that the use of a new pair of gloves to handle each individual is a good method for preventing the transmission of pathogens, best practice should clearly be to handle larval amphibians using a new pair of gloves for each individual, of a type known to have no negative effects on that species. This can easily be done by initially determining which species are susceptible to which types or brands (or even batches) of gloves. Researchers can take a few simple steps to ensure that the gloves they use are not toxic to the amphibians they handle. We recommend a two-phase approach. If researchers have been handling larvae with disposable gloves in captivity or in a situation where individual larvae have been observed for 24 hours following contact and no adverse effects have been seen, then researchers should continue using those same gloves. However, they should remain vigilant for any unusual mortality by following the second phase of the following protocol.

First, researchers should conduct a simple experiment in which tadpoles or salamander larvae of a given species are handled using the gloves in question and then observed for 24 h for signs of morbidity or mortality. Sample sizes do not need to be especially large. Cashins et al. (2008) found that when gloves are toxic, ≥ 40% of the individuals were affected. Using 10% as a more conservative figure for the maximum number of individuals that may experience negative effects, a sample size of 29 provides a 95% probability of observing at least one morbid or dead animal if the gloves under examination have deleterious effects on the species being tested. For rare and endangered species, replicate individuals could be handled and observed sequentially so that individuals are not unnecessarily killed; a single death would suggest a problem with the gloves. It is important that each animal be handled with new gloves and housed in a separate container so that individuals are independent replicates. Note that plastic containers can leach bioactive contaminants (McDonald 2008), so it is worth scrutinizing containers too. Researchers should also use controls, in this case individuals handled with clean bare hands or with gloves that have been demonstrated to be safe with the species in question. Since there is no way to predict *a priori* which species will be affected

TABLE 2. Glove related contact and associated morbidity and mortality for amphibian larvae handled in field settings where the observation periods post-handling would have permitted detection of effects described by Cashins et al. (2008). These results are from ponds where animals could 1) be easily observed in the pond shallows for a period of time (>6 hours) post-glove contact (OBS); 2) where animals were resampled over the course of the season with a high recapture probability (RCAP); or 3) where the protocol required holding animals for observation (> 2 hours) after processing prior to the release of the animals back into the pond (HOLD).

Species	Origin	N	Contact type[1]	Contact duration	Type of gloves used[2]	Monitoring	Response[3]	Reference
Wood Frog (*Rana sylvatica*)	Northwest Territories, Canada	32	SVL CLIP SWAB	1–2 min	P-LTX P-NIT	OBS	None observed	D. Schock (pers. comm.)
Western Toad (*Bufo boreas*)	Northwest Territories, Canada	98	SVL CLIP SWAB	1–2 min	P-LTX P-NIT	OBS	None observed	D. Schock (pers. comm.)
Boreal Chorus Frog (*Pseudacris maculata*)	Northwest Territories, Canada	4	SVL CLIP SWAB	1–2 min	P-LTX P-NIT	OBS	None observed	D. Schock (pers. comm.)
Arizona Tiger Salamander (*Ambystoma tigrinum nebulosum*)	Arizona, USA	2309	VIE CLIP	2 min	P-LTX N-LTX	RCAP HOLD	None observed	Greer et al. (2009)

[1] SVL = snout–vent length measurement, STAGE = developmental stage, CLIP = tissues sample, SWAB = swab for DNA, VIE = visible implant elastomer tag marking.
[2] P-LTX = powdered latex, N-LTX = non-powdered latex, P-NIT = powdered nitrile
[3] Based on description of morbidity and mortality described by Cashins et al. (2008)

by which gloves, this experiment should be conducted with every species to be handled using each type (e.g., latex, nitrile, or vinyl) and brand of glove that will be used. If it is impossible to hold individuals of a given species for 24 h, a sister species may be used. Given the potential for batch-to-batch variation in the manufacturing process, it may be preferable to order large batches of gloves after finding one that does not seem to cause mortality.

Second, once a suitable type and brand of glove are identified, experiments should be used intermittently to test new batches of these gloves for safety. As confidence in the safety and quality control of the product is established then the testing could be less frequent.

There are several other simple steps that researchers should take to minimize the risk to amphibians. Cashins et al. (2008) found that rinsing vinyl gloves in water reduced their toxicity, so any protocol should include rinsing gloves in fresh water prior to handling animals. The water used for this purpose could be collected on site but should be discarded terrestrially. We also recommend researchers pay attention to where gloves are stored to avoid unnecessary risks of gloves coming within close proximity of volatile toxic substances. While we cannot control how and where gloves are stored before we receive them, we can avoid storing them near disinfectants, insecticides, or laboratory chemicals. Lastly, we as a community should watch for and report morbidity and mortality associated with handling tadpoles and larvae in the lab or field. These findings should be openly shared with others who work on amphibians in order to avoid unnecessary morbidity and mortality. Disposable gloves are an essential component of any animal hygiene protocol and by taking these simple steps researchers can ensure that they help, rather than harm, the animals we study.

TABLE 3. Amphibian species at the Detroit Zoo that have been repeatedly handled using gloved hands and otherwise exposed to gloves during husbandry and veterinary care while in the larval lifestage. All animals are checked daily as part of routine care and husbandry. For several years, powdered latex gloves have been primarily used although infrequently non-powdered nitrile gloves are used. In no instance has glove-related morbidity or mortality as described by Cashins et al. (2008) ever been detected. Reference for all information in Table 3 is R. Johnson and D. Schock, personal observations.

Species	Origin[1]	N[2]
Puerto Rican Crested Toad (*Bufo lemur*)	CAPT-DZ	TENS
Wyoming Toad (*Bufo baxteri*)	CAPT-DZ	TENS
Panamanian Golden Frog (*Atelopus varius zeteki*)	CAPT-DZ	TENS
Vietnamese Mossy Frog (*Theloderma corticale*)	CAPT-DZ	TENS
Hourglass Treefrog (*Hyla ebraccata*)	CAPT-DZ	TENS
Red-eyed Treefrog (*Agalychnis callidryas*)	CAPT-DZ	TENS
African Clawed Frog (*Xenopus laevis*)	CAPT-DZ	TENS
Golfodulcean Dart Frog (*Phyllobates vittatus*)	CAPT-DZ	ONES
Green and Black Dart Frog (*Dendrobates auratus*)	CAPT-DZ	ONES
Blue Dart Frog (*Dendrobates azureus*)	CAPT-DZ	ONES
Emperor Newt (*Tylototrition shanjing*)	CAPT-DZ	TENS
Anderson's Newt (*Echinotrition andersoni*)	CAPT-DZ	TENS
Axolotl (*Ambystoma mexicanum*)	CAPT-DZ	TENS
Marbled Salamander (*Ambystoma opacum*)	CAPT-DZ	TENS
Texas Blind Salamander (*Eurycea rathbuni*)	CAPT-DZ	TENS
Texas Salamander (*Eurycea neotenes*)	CAPT-DZ	TENS
Red-spotted Newt (*Notophthalmus v. viridescens*)	CAPT-DZ	TENS

[1]CAPT-DZ = captive bred at the Detroit Zoo

[2]Dependent upon the breeding biology of the species. TENS = 10's to 100's of tadpoles surviving through metamorphosis and beyond per breeding event for multiple years, ONES = 1–2 eggs laid per animal's breeding event with several adults producing tadpoles that survive beyond metamorphosis for multiple years.

LITERATURE CITED

BRUNNER, J. L., K. RICHARDS, AND J. P. COLLINS. 2005. Dose and host characteristics influence virulence of ranavirus infections. Oecologia 144:399–406.

CASHINS, S. D., R. A. ALFORD, AND L. F. SKERRATT. 2008. Lethal effects of latex, nitrile, and vinyl gloves on tadpoles. Herpetol. Rev. 39:298–301.

DASZAK P., A. A. CUNNINGHAM, AND A. D. HYATT. 2001. Draft guidelines for international translocation of amphibians with respect to infectious diseases. Attachment 6. *In* R. Speare and Steering Committee of Getting the Jump on Amphibian Disease. Developing management strategies to control amphibian diseases: Decreasing the risks due to communicable diseases. School of Public Health and Tropical Medicine, James Cook University, Townsville. 2001:150–156. Available for download at: http://www.jcu.edu.au/school/phtm/PHTM/frogs/adms/attach6.pdf.

GREER, A. L., M. BERRILL, AND P. J. WILSON. 2005. Five amphibian mortality events associated with ranavirus infection in south central Ontario, Canada. Dis. Aquat. Org. 67:9–14.

———, C. J. BRIGGS, AND J. P. COLLINS. 2008. Testing a key assumption of host-pathogen theory: Density and disease transmission. Oikos 117:1667–1673.

———, J. L. BRUNNER, AND J. P. COLLINS. 2009. Spatial and temporal patterns of *Ambystoma tigrinum* virus (ATV) prevalance in tiger salamanders, *Ambytoma tirgrinum nebulosum*. Dis. Aquat. Org. 85:1–6.

GUTLEB, A. C., M. BRONKHORST, J. H. J. VAN DEN BERG, AND A. J. MURK. 2001. Latex laboratory-gloves: An unexpected pitfall in amphibians toxicity assays with tadpoles. Environ. Toxicol. Pharmacol. 10:119–121.

LYNCH, M. 2001. Amphibian quarantine protocols Melbourne Zoo. Attachment 6. *In* R. Speare and Steering Committee of Getting the Jump on Amphibian Disease. Developing management strategies to control amphibian diseases: Decreasing the risks due to communicable diseases. School of Public Health and Tropical Medicine, James Cook University, Townsville. 2001:157–161. Available for download at: http://www.jcu.edu.au/school/phtm/PHTM/frogs/adms/attach6.pdf.

MCDONALD, G. R., A. L. HUDSON, S. M. J. DUNN, ET AL. 2008. Bioactive contaminants leach from disposable laboratory plasticware. Science 322:917.

MENDEZ, D., R. WEBB, L. BERGER, AND R. SPEARE. 2008. Survival of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* on bare hands and gloves: Hygiene implications for amphibian handling. Dis. Aquat. Org. 83:97–104.

SOBOTKA, J. M., AND R. G. RAHWAN. 1994. Lethal effect of latex gloves on *Xenopus laevis* tadpoles. J. Pharmacol. Toxicol. Methods 32:59.

WEBB, R., D. MENDEZ, L. BERGER, AND R. SPEARE. 2007. Additional disinfectants effective against the amphibian chytrid fungus *Batrachochytrium dendrobatidis*. Dis. Aquat. Org. 74:13–16.

YOUNG, S., L. BERGER, AND R. SPEARE. 2007. Amphibian chytridiomycosis: Strategies for captive management and conservation. Int. Zoo. Yearbook 41:1–11.