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
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>
<thead>
<tr>
<th>Species</th>
<th>Origin</th>
<th>Contact</th>
<th>Type of gloves used</th>
<th>Duration</th>
<th>Type of monitoring</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wood Frog (Rana sylvatica)</td>
<td>Northwest Territories, Canada</td>
<td>PLIX</td>
<td>1-2 min</td>
<td>PLIX</td>
<td>D. Schrock (pers. comm.)</td>
<td></td>
</tr>
<tr>
<td>Western Toad (Bufo boreas)</td>
<td>Northwest Territories, Canada</td>
<td>PLIX</td>
<td>1-2 min</td>
<td>PLIX</td>
<td>D. Schrock (pers. comm.)</td>
<td></td>
</tr>
<tr>
<td>Breeding Treefrog (Pseudacris regilla)</td>
<td>U.S.A.</td>
<td>N-PLIX</td>
<td>2 min</td>
<td>N-PLIX</td>
<td>D. Schrock (pers. comm.)</td>
<td></td>
</tr>
<tr>
<td>Arizona Treefrog (Anaxyrus woodhousii)</td>
<td>Arizona, USA</td>
<td>PLIX</td>
<td>1-2 min</td>
<td>PLIX</td>
<td>D. Schrock (pers. comm.)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Glove-related contact and associated morbidity and mortality for amphibian larvae handled in field settings where the observation period post-handling would have been extended beyond the periods that are currently required including animals for observation (2 hours after processing).
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.

LITERATURE CITED


