presented an image of this species from Pulau Malawali.

***Chrysopelea paradisi*:** ZRC 2.3599, Pulau Banggi (no further sampling data).

***Dendrelaphis caudolineatus*:** ZRC 2.6560 (*ex* ID 7777), west coast of Pulau Balambangan (07°12'N, 116°45'E), 18 June 2003.

FAMILY CROCODYLIDAE

***Crocodylus porosus*:** Not collected, but reported by the local Bajau tribesmen as occurring off the waters of Pulau Balambangan.

Excluding marine turtles, for which no data are currently available, Pulau Banggi and adjacent islands are home to at least eight species of amphibians and 12 species of reptiles. All, save one (*Gekko gecko*), are widespread across the lowlands of Borneo, a distributional pattern similar to that shown by the island group's avifauna (Chasen and Boden Kloss 1930). The sole reptile not known from Borneo is widely distributed in the Philippine Archipelago, and may either be a recent emigrant via the Palawan-Balabac corridor, or competitively excluded on the main island by the closely related and presumably ecologically similar *Gekko smithii*. The islands to the north of the Pulau Banggi group lie within the jurisdiction of the Philippines, and the herpetofaunas of Palawan and Balabac has been enumerated by Mocquard (1890) and Boulenger (1894).

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Lethal Effect of Latex, Nitrile, and Vinyl Gloves on Tadpoles

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Tadpoles are studied in a variety of fields including husbandry, developmental physiology, toxicity testing, and basic biological and ecological research. In many instances it is necessary to use gloves when handling tadpoles or during water changes to protect the experimenter (e.g. teratology research) or to promote hygiene and prevent the transfer of pathogens between tadpoles (Retallick et al. 2006; Sobotka and Rahwan 1999). While investigating aspects of the virulent amphibian fungal pathogen *Batrachochytrium dendrobatidis*, we discovered that a variety of gloves can be lethal to tadpoles. We present here two case studies, one in the lab and one in the field, and two experiments, all demonstrating the lethal effect of gloves on tadpoles. Following exposure to the various glove treatments, all tadpoles were categorized as either fine, listless, or dead.

Case Study 1: Laboratory

Batrachochytrium dendrobatidis infects the skin of frogs, but only the mouthparts of tadpoles (Knapp and Morgan 2006; Marantelli et al. 2004). During a laboratory experiment investigating *B. dendrobatidis* infection in *Litoria genimaculata* and *Litoria nannotis* tadpoles, each tadpole was to be measured, weighed, and its mouthparts swabbed with a sterile cotton swab to test for *B. dendrobatidis* by diagnostic PCR (Boyle et al. 2004). A new pair of latex gloves (SuperMax, low powder) were worn when handling each tadpole to prevent transmission of *B. dendrobatidis* between individuals and to prevent DNA contamination of swab samples. Each tadpole was scooped out of its container with a gloved hand. The tadpole was secured, ventral surface up, in between the index and middle fingers by gently depressing the thumb to the base of the tail. A swab was gently passed over the mouthparts repeatedly to collect *B. dendrobatidis* DNA on the cotton fibers. Each tadpole was in hand for approximately 30–90 seconds before being returned to its container.

Thirty-six *L. genimaculata* had been processed in this way when we observed that some of the earliest handled tadpoles appeared listless, could not remain upright, and had difficulty using their tails for locomotion. Upon closer inspection, the tails of the listless tadpoles were gray and dead in appearance at the locations where gloved fingers held them in place during swabbing. At that time, we suspended tadpole handling. Within 24 h, 26 of the 36 tadpoles died. The surviving ten tadpoles did not appear listless, showed no overt adverse effects and survived 4–6 weeks to metamorphosis (Fig. 1A). Although care was taken to handle tadpoles gently, the observed mortality could possibly have been due to mechanical damage, so we initiated a series of experiments. Based on the results of these experiments (see below), we switched from latex to vinyl gloves for the remainder of the lab study. Ten unhandled *L. genimaculata* and *L. nannotis* tadpoles were processed as previously described except with vinyl instead of latex gloves. Following 24 h of observation, no mortality or ill effects were noted. Satisfied that vinyl gloves were safe for tadpoles of these species, the remaining 13 *L. genimaculata* and 22 *L. nannotis* tadpoles were processed using vinyl gloves (Fig. 1A). All tadpoles appeared unaffected after handling. In total, 26/36 *L. genimaculata* died following handling with latex gloves, while 0/23 *L. genimaculata* and 0/32 *L. nannotis* died following contact with vinyl gloves.

Experiment 1: Glove Soak

To determine the best gloves for handling tadpoles, we conducted an experiment testing the three most common glove types: latex (SuperMax, low powder), vinyl (Livingstone, clear, low powder), and nitrile (Livingstone, low powder). Forty non-native *Bufo marinus* tadpoles were captured from a local pond and allowed to rest in individual 1000 mL containers with 500 mL of collected rainwater and a pinch of powdered tadpole chow (3:1 alfalfa pellets:fish food, ground and passed through a 250 μm sieve). After 24 h, one of each glove type was draped over the edge of the container for five minutes so that the five fingers of each glove were submerged. Ten control containers had no contact with a glove. The condition of each tadpole was recorded at 2, 12, 24, and 72 h following removal of the glove, however, tadpole condition did not change beyond the two hour post-exposure point. One of ten tadpoles exposed to the latex gloves died and two more were listless and floating awkwardly within two hours of glove exposure. One of the tadpoles exposed to nitrile gloves was listless while all of the vinyl and control treatments appeared unaffected (Fig. 1B). The listless tadpoles remained in an impaired state for the full 72 h of observation and appeared permanently affected. These listless tadpoles were euthanized and preserved in 70% ethanol. Although three of the ten *B. marinus* tadpoles exposed to latex gloves experienced deleterious effects, the rate of mortality was lower than we expected given the high level of mortality we previously observed in *L. genimaculata*. We hypothesized this difference was related to the different methods of glove exposure. The *L. genimaculata* in "Case Study 1" were in direct physical contact with the gloves during measuring and swabbing while the *B. marinus* in "Experiment 1" were in water in which gloves were soaked. This indirect glove contact may have yielded a lower dose of the toxic compound(s).

Experiment 2: Glove Contact

To determine whether direct glove contact increases mortality, we ran a second experiment in which *B. marinus* tadpoles were handled in the same manner as previously described for *L. genimaculata* and *L. nannotis*. Each tadpole was gently held in place at the base of the tail between the thumb and index finger for 60 seconds with one of the three types of gloves as treatments or with an ungloved hand as a control. A new glove was worn for each tadpole and the treatments were interspersed, with each glove type and the bare hand treatment applied in succession. Prior to the no glove treatment, hands were rinsed in tap water and dried with a paper towel to remove any residual powder from the previous glove treatment.

Within two hours of handling, all tadpoles that had been in contact with latex or nitrile gloves were dead or listless. Those that were listless died within 24 h (Fig. 1C). Listless tadpoles had little to no tail function and the usually dark black tail had a discolored, dead-looking, gray appearance. This discoloration was most pronounced where direct contact with the gloves occurred. Particles in the water soon began to attach to the epidermis of the dying tail, giving it a fuzzy appearance. None of the tadpoles handled with either vinyl gloves or bare hands suffered noticeable ill effects and all survived to metamorphosis (Fig. 1C).

Case Study 2: Field

We applied our conclusion that vinyl is the safest glove material to "Case Study 1: Lab" (described above) as well as a field study monitoring *B. dendrobatidis* in the wild. Individual tadpoles were to be captured, handled with vinyl gloves, measured, swabbed for *B. dendrobatidis* infection, and returned to the stream unharmed. During initial field sampling, individuals were processed and kept temporarily in a holding tray to monitor condition following swabbing. Unexpectedly, of the first ten *L. nannotis* tadpoles processed, four became listless and died within one hour (Fig. 1D). The remaining six tadpoles appeared normal and did not develop signs over the following 24 h. As a test, the next ten captured tadpoles were processed with bare hands and suffered no ill effects, suggesting the gloves and not the handling were the cause of mortality. The next ten captured tadpoles were processed with vinyl gloves that were rinsed in a bucket of water prior to handling. All of these tadpoles survived and appeared normal suggesting that a substance on the outside of the vinyl glove was toxic and that rinsing successfully removed it. All tadpoles were held for 24 h for observation. From this point on we incorporated the rinsing of vinyl gloves into the standard field protocol. Vinyl gloves were rinsed in a 10 L bucket of water which was changed after at most ten tadpoles. This was adequate to ensure the glove-wash residue did not attain a high enough concentration to cause harm. To date over 2500 tadpoles have been handled with washed vinyl gloves with no ill effects. On a few occasions, the rinsing step was accidentally skipped and many of these tadpoles became listless and died.

The fact that the same type and brand of vinyl glove did not cause mortality in *L. nannotis* tadpoles in the laboratory trials but did cause mortality in the field suggests that the presence or level of the toxic compound(s) may vary among boxes of gloves. This may be a result of varying conditions during glove fabrication. During production of disposable gloves, a large number of chemicals are added including vulcanizers, accelerators, colorants, preservatives, stabilizers, and antistatic agents (Boman et al. 2004). These chemicals are typically the cause of glove sensitivity in humans. The type and quantity of these compounds can vary widely among manufacturers and possibly even production runs (Boman et al. 2004).

Our results show that unwashed latex, nitrile, and vinyl gloves can be toxic to tadpoles. Unwashed latex and nitrile gloves caused up to 100% tadpole mortality following only 30–90 seconds of direct contact (Fig. 1C). Rapid, localized necrosis of tissue at the point of contact was observed grossly. Even five minutes of partial glove submersion was sufficient to cause mortality in the latex and nitrile treatments (Fig. 1B).

Despite a thorough literature search, only two references to the toxic effects of gloves on tadpoles were found and both of these were published in toxicological journals, likely to have low readership by herpetologists. In a letter to the editor, Sobotka and Rahwan (1999) reported that water from unwashed latex gloves (American Dental Association, Safeskin brand) and washed latex gloves (Baxter Pharmaseal Flexam) soaked for 24 h caused mortality in *Xenopus laevis* tadpoles. However, water from washed vinyl gloves (Baxter Triflex) did not. Gutleb et al. (2001) reported 100% mortality in *X. laevis* and *Rana temporaria* tadpoles exposed to water from unwashed latex gloves (Becton-Dickinson) soaked for 24 h. Even very dilute solutions of glove-soaked water (0.29% for *X. laevis* and 0.15% for *R. temporaria*) caused 100% mortality. Gutleb et al. (2001) found that vinyl gloves (Becton-Dickinson) soaked for 24 h also killed tadpoles, but only at relatively high concentrations: 33% and above. Mortality was 100% at or above this concentration but 0% below this concentration.

Our results, together with the results from these published studies, demonstrate the potentially high toxicity of latex gloves to tadpoles. Different brands of latex gloves, different exposure methods, and tadpoles of different species were used in each study. Sobotka and Rahwan (1999) tested washed and unwashed latex gloves. The end result, however, was the same: significant

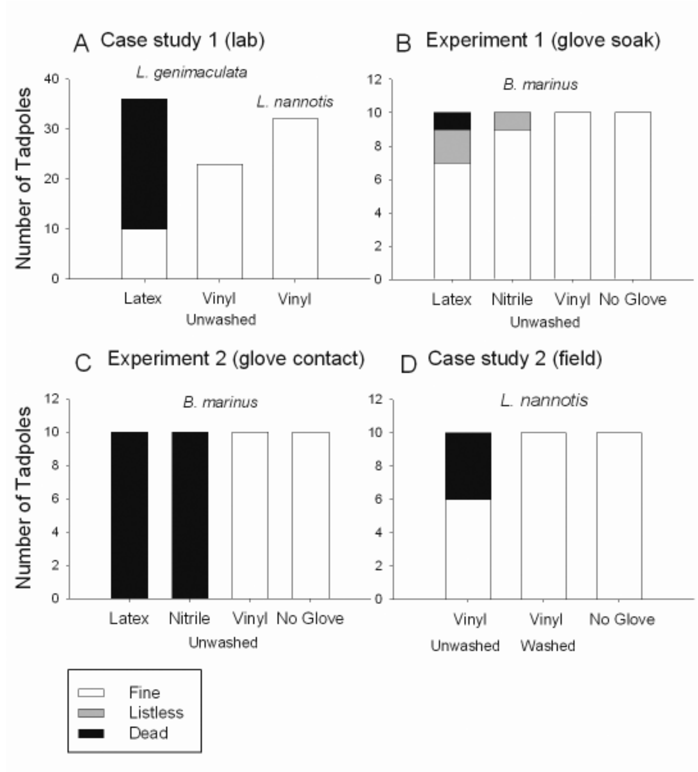

FIG. 1. A) Case Study 1: Laboratory. Number of *Litoria genimaculata* and *Litoria nannotis* tadpole deaths within 24 h following contact with latex or vinyl gloves for 30–90 seconds. B) Experiment 1: Glove Soak. Number of *Bufo marinus* tadpole deaths within 72 h following contact with water containing latex, vinyl, and nitrile gloves soaked for 5 minutes. C) Experiment 2: Glove Contact. Number of *B. marinus* tadpole deaths within 24 h following direct contact with latex, vinyl, and nitrile gloves and bare hands for 60 seconds. D) Case Study 2: Field. Number of *L. nannotis* deaths within 24 h following direct contact with unwashed vinyl gloves, washed vinyl gloves, and bare hands for 30–90 seconds.

tadpole mortality. This suggests that glove toxicity may be associated with many different brands of disposable latex glove and tadpoles of many different species are likely to be affected. Ours is the first report that nitrile gloves can also be extremely toxic to tadpoles, producing 100% mortality in *Bufo marinus* following direct glove contact.

We found that unwashed vinyl gloves can also cause mortality, however, at a lower rate than either latex or nitrile gloves. This finding is supported by Gutleb et al. (2001) who found that vinyl glove-soaked water caused mortality only at dilutions over 110 times more concentrated than latex glove soaked water. Importantly, by rinsing the vinyl gloves in water we eliminated any obvious toxicity.

As a result of the apparently more toxic nature of latex and nitrile gloves compared with vinyl, and the ability to eliminate toxicity in vinyl gloves through rinsing, we recommend the use of well rinsed vinyl gloves when handling tadpoles or cleaning aquaria. However, all glove brands and types are potentially toxic and should not be used until proven safe with tadpoles of the particular species being handled. Even then, handled tadpoles should be observed carefully as toxicity may vary between production runs.

It is important to note that gloves have not been found to affect juvenile or adult amphibians negatively. The use of gloves to handle amphibians is widespread in the field and lab. Changing gloves between amphibians remains an important hygiene measure to prevent transmission of infectious agents such as *B. dendrobatidis* and ranaviruses between individual amphibians and aquaria. However, given our tadpole results, it would be useful to investigate potential non-lethal effects of gloves on adult and juvenile amphibians to ensure that gloves really are entirely non-injurious.

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TECHNIQUES

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Visible Implant Fluorescent Elastomer: A Reliable Marking Alternative for Snakes

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Studies in population ecology require use of reliable marking techniques to estimate various parameters (e.g., population size, density, demographics, movement, or behavior; Penney et al. 2001; Perret and Joly 2002; Walsh and Winkelman 2004; Woods and Martin-Smith 2004). However, it is imperative that marking techniques meet standard assumptions: 1) marks must remain visible for the duration of the experiment, 2) marks are correctly recorded, 3) marks do not affect the survival of the animal, and 4) marks do not affect the recapture probability of animals (Goldsmith et al. 2003; Otis et al. 1978).

Visible implant fluorescent elastomer (VIE; Northwest Marine Technology, Inc., Shaw Is., Washington, USA) was initially developed for batch marking migratory fish, but has recently been used to mark amphibians and lizards (Bailey 2004; Losos et al. 2004; Nauwelaerts et al. 2000; Nishikawa and Service 1988; Penney et al. 2001). Visible implant fluorescent elastomer consists of a liquid polymer added to a curing agent to create a flexible plastic mark. Color kits are available, capable of marking 15,000 individuals depending on the number of colors used and marking design. Our objective was to determine if VIE was an appropriate marking technique for snake research based on the marking assumptions of Otis et al. (1978) and Goldsmith et al. (2003). We hypothesized that VIE would be a reliable marking technique for snakes. To our knowledge, our study is the first to apply VIE to snakes.

We conducted this empirical study in a laboratory setting at North Carolina State University, Raleigh, North Carolina, USA. We marked Red Cornsnakes (*Pantherophis guttatus*; N = 18) between 19 and 29 April 2006. Each snake received three doses (1, 2, and 3 μl) of yellow VIE randomized to the general area of three locations (neck, midbody, and pre-caudal). We injected marks subcutaneously and dorsolaterally on left sides using a graduated 1cc Luer-lok syringe with a 25-gauge needle (Becton-Dickinson, Franklin Lakes, New Jersey, USA). We used 1cc syringes to better approximate volumes, which required the 25-gauge needle for a secure fit. We injected additional *P. guttatus* (N = 4) and Common Kingsnakes (*Lampropeltis getula*; N = 6) with