Copeia, 2003(3), pp. 601–607

Pathogenicity and Transmission of Chytridiomycosis in Tiger Salamanders (*Ambystoma tigrinum*)

Elizabeth W. Davidson, Matthew Parris, James P. Collins, Joyce E. Longcore, Allan P. Pessier, and Jesse Brunner

A chytrid fungus, *Batrachochytrium dendrobatidis*, was found in salamanders, *Ambystoma tigrinum stebbinsi*, collected in southern Arizona, USA. The chytrid was isolated and cultured, and Koch's postulates were satisfied by infection of metamorphosed salamanders with pure culture and subsequent reisolation from these salamanders. We used the salamander strain and a strain isolated from lowland leopard frogs in Arizona, *Rana yavapaiensis*, to infect metamorphosed *A. tigrinum*, *R. yavapaiensis*, and *R. boylii*. All three species became infected, but none of the infected salamanders died within 60 days, and mortality of infected frogs did not differ significantly from controls, although sample size was small. Chytrid infection could not be detected by light histology in most of the infected frogs and one of the infected salamanders 60 days after infection. To date, there are three records of chytridiomycosis in salamanders on websites; ours is the first complete report of occurrence and pathology of chytridiomycosis in field-collected North American salamanders. Our results also demonstrate that chytridiomycosis does not always lead to mortality. Individuals within a species vary in susceptibility to infection, animals appear to recover from the infection, and syntopic salamanders and frogs may act as reciprocal pathogen reservoirs for chytrid infections.

PATHOGENS, including viruses and fungi, are among the suspected causes of global amphibian declines (Daszak et al., 1999; Carey et al., 2003; Collins et al., 2003). A pathogenic, chytrid fungus (*Batrachochytrium dendrobatidis*) is implicated in the declines of frog populations in North America, South America, Europe, and Australia (Berger et al., 1998; Bosch et al., 2001). We report the first case of chytrids infecting salamanders (*Ambystoma tigrinum*) collected in the field. Amphibian communities are often mixtures of frogs and salamanders, raising the possibility that either might serve as a pathogen reservoir for the other. Reservoir species often play an important role in sustaining epidemics as they harbor virulent pathogens in the intervals between host infections. The continued transmission of pathogens from reservoirs to more susceptible species is thought to play an especially important role in pathogen-driven extinctions.

During routine sampling of the Sonora tiger salamander (*Ambystoma tigrinum stebbinsi*) in November and December of 1999 in the San Rafael Valley in southern Arizona, we noted that 27 of 54 metamorphosed animals had small black spots on their ventral abdomen and dorsal head surface. We brought five of the black-spotted salamanders to the laboratory for further study. Assays with fish cell culture revealed no iridovirus from tail clips (Jancovich et al., 1997). Skin sheds, however, had profiles consistent with infection by *B. dendrobatidis*, including phase-bright spherical colonial sporangia with cross-membranes (Longcore et al., 1999).

In Arizona, *B. dendrobatidis* was implicated in mortality of native Chiricahua (*Rana chiricahuensis*) and lowland leopard frogs (*Rana yavapaiensis*), and the canyon treefrog, *Hyla arenicolor*, in 1999 (Bradley et al., 2002) but has not previously been identified from North American urodeles. Furthermore, although correlation of chytrids with loss of anuran populations is well documented, the pathogenicity of the fungus to urodeles is unknown.

Because of the increasing evidence for amphibian population declines correlated with chytrid infections (Carey et al., 2003), it is critical to understand the pathology and fitness consequences of infection in all affected amphibian taxa. We isolated *B. dendrobatidis* from field-collected *A. tigrinum*, cultured it, and performed a series of cross-species exposure laboratory experiments to test the pathogenicity of the salamander-derived fungus in *A. tigrinum*, *R. yavapaiensis*, and *Rana boylii* (foothill yellow-legged frogs). *Rana yavapaiensis* population declines in Arizona are often associated with chytrid infections (D. E. Green, unpubl., cited in Daszak et al., 1999; Bradley et al., 2002). *Rana boylii* is endemic to the western United States. In a reciprocal test, we inoculated the same three species with *B. dendrobatidis* isolated from *R. yavapaiensis* from Arizona. We addressed

three questions: (1) Do frog and salamander species differ in chytrid susceptibility? (2) Are anuran species with different histories of exposure to chytrid differentially susceptible? (3) Do isolates of *B. dendrobatidis* from salamanders and anurans differ in pathogenicity?

Materials and Methods

Fungal isolation and culture.—Four recently metamorphosed, laboratory-reared *A. tigrinum* were housed for 13 days at 25 C in water that had housed one of the field-collected, chytrid-infected salamanders. The water was replaced with fresh water after the 13-day exposure. Three salamanders died after 13, 35 and 46 days of exposure. After 40 days, we euthanized the fourth salamander, which was fixed for light and electron microscopy. We isolated *B. dendrobatidis* from skin from the ventral body wall of the salamander that died on day 13.

We cleaned yeasts and bacteria from small pieces of infected skin of *A. tigrinum* by wiping through mTGhL agar (8 g tryptone, 2 g gelatin hydrolysate, 4 g lactose, 10 g agar, 1 liter distilled water) with a sterile needle. Skin pieces were then placed on plates containing mTGhL agar, to which 200 mg penicillin-G and 400 mg streptomycin sulfate were added after autoclaving. Isolation plates were incubated at 23 C. A pure culture was obtained and designated isolate A-277. This isolate is morphologically similar to *B. dendrobatidis* (Longcore et al., 1999).

Rana yavapaiensis were collected during a mortality event in January 1999, in Montrose Canyon, Santa Catalina Mountains, southern Arizona by M. Sredl, Arizona Game and Fish Department (Bradley et al., 2002). Chytridiomycosis was confirmed both histologically and by isolation of *B. dendrobatidis*, and this isolate (R-230) was used in cross-transmission studies. Isolates were cultured on TGhL nutrient agar (16 g tryptone, 4 g gelatin hydrolysate, 2 g lactose, 10 g agar, 1 liter distilled water) in 9 cm culture plates. Cultures were passed five times or fewer before use in our experiments.

To harvest zoospores for inoculation trials, we added 3 mL of sterile water to two-week-old fungal cultures. After about 30 min the water was dense with zoospores that emerged from the fungal zoosporangia. Zoospores were counted using a hemocytometer.

Infection of salamanders and frogs with cultured zoospores.—To observe the progression of *B. dendrobatidis* infection in *A. tigrinum*, we attempted infection of four laboratory-reared, metamorphosed juvenile and three field-collected, adult metamorphosed salamanders. We histologically examined two toe clips collected from each salamander 14 days before exposure. The epidermal skin of hematoxylin- and eosin-stained sections of toe clips contained no *B. dendrobatidis* thalli.

We randomly assigned the seven salamanders, varying in size and age, to control or treatment groups. Salamanders were placed in individual containers with 1 L aged tap water and held at 18 C. Three salamanders received water bath exposure to isolate A-277 (9×10^3 zoospores/mL), two were exposed to isolate R-230 (6×10^3 zoospores/mL), and two received no zoospores (controls). Water was changed one week after exposure. Salamanders were fed crickets twice weekly, and water was changed weekly. Shed skin was collected at each water change, fixed, and examined microscopically for *B. dendrobatidis*. On day 15, one salamander exposed to isolate A-277 was euthanized and fixed for light and electron microscopy. Remaining animals were observed for morbidity and mortality for 60 days, after which they were euthanized and fixed for light and electron microscopy.

We then investigated the susceptibility of native frog species to the chytrid isolated from salamanders. *Rana yavapaiensis* larvae were collected from an Arizona State University pond facility and reared in the laboratory in conspecific groups through metamorphosis. *Rana boylii* were collected as larvae from the South Fork of the Eel River, near Laytonville, California (39°43′07″N, 123°39′10″W) by Dr. Carlos Davidson (California State University-Sacramento) and reared through metamorphosis.

Ten metamorphosed *R. yavapaiensis* and 37 metamorphosed *R. boylii* were acclimated at 22 C for 20 days in 470 mL Zip Lock® (S. C. Johnson, Racine, Wisconsin) containers with 150 mL water and then randomly assigned to treatment groups as follows (Table 1). Four *R. yavapaiensis* each were exposed to 8.5×10^3 zoospores/mL of R-230 or A-277. Eight *R. boylii* each were exposed to 8.5×10^3 zoospores/mL of R-230 or A-277, and seven *R. boylii* each were exposed to 8.5×10^2 zoospores/mL of R-230 or A-277. Zoospores were introduced into the water surrounding the frogs. Three *R. yavapaiensis* and seven *R. boylii* were kept as controls (Table 1). Frogs were held at 22 C for 60 days, fed crickets twice weekly, and water was changed weekly. Frogs were weighed prior to treatment and survivors were weighed 60 days after exposure. Dead animals were immediately fixed in 10% formalin, and after 60 days all remaining experimental and control frogs were euthanized and fixed in formalin for histology. Responses mea-

TABLE 1. NUMBER OF DEATHS, TIME TO DEATH AND WEIGHT GAIN OF SURVIVING *Rana yavapaiensis* AND *Rana boylii* AFTER INOCULATION WITH *Batrachochytrium dendrobatidis* ISOLATED FROM EITHER *Rana yavapaiensis* (R-230) OR FROM *Ambystoma tigrinum* (A-277).

Test species	Isolate and zoospores/ml	No. dead/ No. dosed	Growth of survivors (× mg ± SE)	Time to death (× day ± SE)
R. yavapaiensis	R-230 @ 8.5×10^3	3/4	1147	26.3 ± 4.7
	A-277 @ 8.5×10^3	1/3	1289 ± 690	7
	Control	1/3	985 ± 488	24
R. boylii	R-230 @ 8.5×10^3	3/8	1056 ± 161	53.3 ± 9.5
	R-230 @ 8.5×10^2	4/7	750 ± 207	22.0 ± 8.3
	A-277 @ 8.5×10^3	3/8	1387 ± 161	31.0 ± 9.5
	A-277 @ 8.5×10^2	2/7	895 ± 161	10.5 ± 11.7
	Control	2/7	947 ± 161	52.0 ± 11.7

sured included survival, time to death and growth of survivors.

Survival data were analyzed with a χ^2 goodness-of-fit test. Time to death and growth rate data were analyzed with ANOVA. Preliminary analyses indicated initial body mass did not have a significant effect on either time to death or growth rate (ANCOVA), thereby justifying the use of ANOVAs.

Light and electron microscopy.—Sloughed skin was collected from all infected animals before each water change and fixed in 2.5% glutaraldehyde in cacodylate buffer for examination with phase-contrast microscopy. Tissues from infected and control animals were fixed for light histology in 10% buffered formalin, and embedded in paraffin. Light histology analysis was based on examination of two cross-sections through the tail and cross-sections of a hind limb and foot. Samples were fixed for electron microscopy in 2.5% glutaraldehyde in cacodylate buffer, or 2.5% paraformaldehyde plus 2.5% glutaraldehyde in Sorenson's phosphate buffer (Dawes, 1971), postfixed in OsO_4, dehydrated, and embedded in Spurr's resin. Thin sections were stained with uranyl acetate and lead citrate and examined using a Philips CM12S electron microscope.

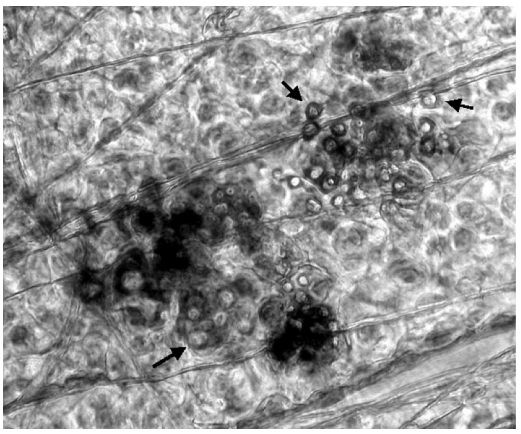

Fig. 1. Sloughed skin from *Ambystoma tigrinum* demonstrating concentration of *Batrachochytrium dendrobatidis* thalli (arrows) within melanized spots. Photographed at 10×.

RESULTS

Chytrid isolation and identification.—Profiles of the organism found in the surface epidermis of field-collected and experimentally infected tiger salamanders were consistent by light microscopy histology with *B. dendrobatidis* (Pessier et al., 1999). Zoospores produced in pure culture were posteriorly uniflagellate with several lipid globules. Rhizoids on thalli were threadlike, and mature zoosporangia had one to several discharge papillae of varying length. Some thalli were colonial, as also noted in descriptions of *B. dendrobatidis* (Longcore et al., 1999; Pessier et al., 1999). We have reisolated *B. dendrobatidis* from two initially pathogen-free salamanders that were infected using pure cultures, thereby satisfying Koch's postulates.

Nine days after exposure to zoospores, chytrid profiles were observed in sloughed skin of all salamanders exposed to either A-277 or R-230. None of the five salamanders exposed to zoospores died during 60 days; one was euthanized for histological examination at 15 days after exposure. Some chytrid-infected salamanders began to slough skin within one hour after exposure to zoospores, and continued to slough repeatedly over the experimental period. Sloughs frequently contained melanized spots as observed on the initial salamanders collected in the field. These spots surrounded clusters of chytrids (Fig. 1).

Thirty-four days after exposure, one salaman-

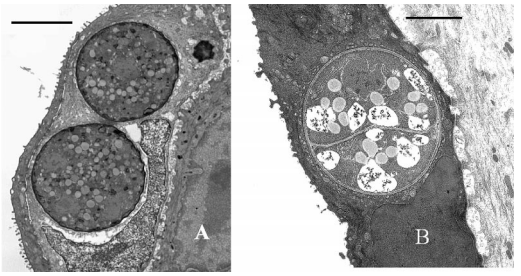

Fig. 2. Developing monocentric (A) and colonial (B) thalli in the keratinized skin layer of *Ambystoma tigrinum*. Electron micrographs. Bar = 4 μm.

der exposed to R-230 ceased to display chytrid profiles in sloughed skin, and two exposed to A-277 displayed greatly reduced chytrid forms. One original field-collected salamander in which chytrids were first observed also showed no evidence of infection after holding in the laboratory for four months, and no chytrid forms were later observed on shed skin of this animal. None of the control animals were infected, and sloughs were produced much less frequently by controls.

Summary of histologic findings in salamanders.— Early changes in the skin of the animal euthanized at 15 days postexposure to A-277 included a mild increase in skin thickness with minimal to mild disorganization of the epidermal layers and mild increase in numbers of epidermal mitotic figures and granulocytic inflammatory cells, and minimal hyperkeratosis (thickening of outermost keratinized layer) of the epidermis with small numbers of chytrid thalli within this layer. At 60 days after start of the experiment, all salamanders exposed to zoospores were positive for chytrids with the exception of one of the two exposed to R-230 that was also negative by analysis of sloughed skin. At 60 days postinoculation, skin was moderately thickened, attributable to a minimal to mild increase in number of cells in the epidermal layer plus an increase in skin cell (keratinocyte) size. Epidermal cell layers were moderately disorganized with less orderly progression from basal cell layers to skin surface. Mitotic figures and granulocytic inflammatory cell infiltrates were prominent. There was minimal to occasional mild orthokeratotic hyperkeratosis (as at 15 days) with similar numbers of chytrid thalli as at 15 days. Histologic lesions in *A. tigrinum* differed slightly from cases of anuran chytridiomycosis (Pessier et al., 1999) in that affected salamanders had mild cutaeous inflammatory cell infiltrates and only minimal to mild hyperkeratosis. Control

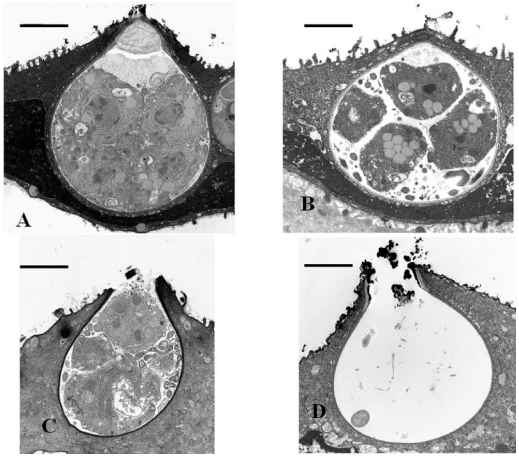

Fig. 3. Progressive stages of development of zoospores in keratinzed layer of the skin of *Ambystoma tigrinum*. Discharge of zoospores leads to characteristic empty zoosporangium (D). Electron micrographs, bar = 1 μm.

animals had low numbers of both mitoses and inflammatory cells, but at levels well below those of animals with identifiable chytrids.

Ultrastructural examination of salamanders exposed to A-277 confirmed the presence of intracellular chytrid thalli at several stages of development (Fig. 2). Characteristic bottle-shaped zoosporangia were observed at all stages of producing zoospores, as well as empty zoosporangia (Fig. 3). Ultrastructural features of A-277 in *A. tigrinum* skin closely resembled those observed in White's treefrog (Pessier et al., 1999).

Skin sloughs collected from chytrid-treated *R. yavapaiensis* and *R. boylii* exhibited bottle-shaped and circular figures with cross-membranes typical of chytrid sporangia as early as four days postexposure to both chytrid strains. Three of four *R. yavapaiensis* exposed to R-230 and one of three exposed to A-277 died, whereas one of three control frogs died during the 60-day exposure period (Table 1). Two *R. yavapaiensis* exposed to R-230 that died at 17 and 31 days postexposure had histological evidence of chytrids; all of the remaining frogs, including controls, were negative.

Seven of 15 *R. boylii* exposed to R-230, and five of 15 exposed to A-277 died, as well as two of seven controls, during 60 days (Table 1). Histology revealed chytrids in two *R. boylii* exposed to 8.5×10^3 zoospores/mL of strain R-230, one that died at 51 days postexposure and one that survived for 60 days. One *R. boylii* exposed to 8.5×10^2 zoospores/ml of strain A-277 that survived for 60 days was also positive. All these animals displayed only mild infec-

tions. *Batrachochytrium dendrobatidis* thalli were not detected in any of the other *R. boylii*. Survival, time to death, and growth rate were not statistically different between treated and control frogs of either species (Table 1), although our power to detect differences was low because of small sample sizes.

Discussion

We report a chytrid infection in field-collected salamanders and transmission of this chytrid in the laboratory to salamanders and frogs. *Batrachochytrium dendrobatidis* thalli have been previously observed in five captive axolotls, *Ambystoma mexicanum*, purchased from pet shops in Australia (R. Speare and L. Berger, 2000, http://www.jcu.edu.au/school/phtm/PHTM/frogs/chyspec.htm), in captive *Salamandra salamandra* from Germany (F. Mutschmann, pers. comm.; R. Speare and L. Berger, 2000, http://www.jcu.edu.au/school/phtm/PHTM/frogs/chyspec.htm) and chytrid was detected by PCR in a dead, field-collected Idaho giant salamander, *Dicamptodon aterrimus* (USGS National Wildlife Heath Center Quarterly Mortality Report, http://www.nwhc.usgs.gov/pub_quarterly/4qt01tbl.html; D. E. Green, pers. comm.). Because axolotls are paedomorphic, infection by chytrids suggests that larval as well as metamorphosed salamanders may be potential chytrid hosts. Transmission of the chytrid isolated from salamanders to frogs and vice versa demonstrates that these *B. dendrobatidis* strains are not host specific.

Some *A. tigrinum* in our experiments harbored extensive chytrid infections without succumbing to the infection. We found evidence of chytrid infection in the sloughed skin of salamanders soon after inoculation, and some, including an original field-collected salamander, showed no signs of infection later in the experiment. The rapid and frequent sloughing response of salamanders may contribute to their ability to reduce or rid themselves of the infection. Frogs also sloughed skin that contained chytrid forms shortly after inoculation but appeared to be little affected by chytrid exposure and most apparently lost their infections. Mortality, time to death, and growth rates of infected frogs were similar to controls. Of the 19 total exposed frogs of both species that died during the 60-day experiment, only one had obvious chytrid infection on histological examination, and only three of the exposed frogs that survived for 60 days exhibited chytrid forms. False negatives are possible during histological examination, but these results suggest that most frogs had greatly reduced or no chytrid present 60 days after exposure. Response to laboratory stress was markedly different for salamanders and frogs as expressed in control mortality of frogs (33% and 28%) versus salamanders (0% in 60 days).

It is striking that a pathogen associated with mortality and declines in many frog species was not more pathogenic under controlled laboratory conditions. In our experiments, mortality of *A. tigrinum*, *R. yavapaiensis*, and *R. boylii* was sporadic and appeared unrelated to dose or strain of chytrid. Even heavy chytrid infections, as detected in sloughed skin, generally did not lead to mortality. We have no evidence that the cultures we used to inoculate amphibians had lost pathogenicity during in vitro passage, because these cultures had been passed less than five times. Nichols and colleagues have found no difference in pathogenicity of the *B. dendrobatidis* type culture over more than five years of in vitro passage (D. Nichols, pers. comm.). Recently, some wild populations of the African clawed frog, *Xenopus laevis*, were found to carry chytrid infections without mortality, whereas die-offs associated with chytridiomycosis were found in other populations (Hopkins and Channing, 2002). Previous experiments showing *B. dendrobatidis* to be highly pathogenic tested only small numbers of one or a few species of recipient animals (Berger et al., 1998; Longcore et al., 1999; Nichols et al., 2001). Chytridiomycosis may be more lethal to the dendrobatid and bufonid species tested in these earlier studies than to ranids. Other factors, such as initial health of the animal (Rollins-Smith, 2001), or stressors, such as pesticide exposure, bacteria, or temperature, have been postulated to contribute to mortality or recovery of the individual (Carey et al., 1999; Davidson et al., 2001; Hopkins and Channing, 2002). However, in the initial description of chytridiomycosis, no other factors aside from *B. dendrobatidis* were associated with mortality (Berger et al., 1998). Because *B. dendrobatidis* infects only the outer keratinized layer of the skin, antimicrobial peptides may be the primary defense of amphibians against these pathogens. Rollins-Smith et al. (2002) showed that *B. dendrobatidis* zoospores are sensitive to antimicrobial peptides produced in the skin of ranid frogs. Antimicrobial peptides have also been found in salamanders, *Plethodon cinereus* (Fredricks and Dankert, 2000), and *A. tigrinum* (L. Rollins-Smith, pers. comm.). The effectiveness of these peptides is temperature-dependent and environmental factors likely influence their synthesis and release (Matutte et al., 2000).

It is not yet known how *B. dendrobatidis* persists in the environment in the absence of amphibians, and no resting spore stage is known (Bradley et al., 2002). Our results demonstrate that salamanders can serve as reservoir hosts for chytrid infection in frogs and vice versa. These reservoirs may allow the pathogen to persist in habitats with small, seasonal populations of amphibians. As we model the dynamics of chytrid-amphibian interactions, this result will be important for understanding epizootics in areas where frogs and salamanders share habitats, as they often do.

Acknowledgments

This research was funded by award DEB-9816645 from the National Science Foundation to J. Collins, A. Storfer and E. Davidson, and IRCEB IBN-9977063 from the National Science Foundation to J. Collins, A. Storfer, E. Davidson, N. Cohen, J. E. Longcore, and collaborators. We are grateful to R. Medville and R. Roberson for assistance with electron microscopy and ultrastructural interpretation. Experiments were performed under IACUC Permit 00-526R; *A. tigrinum* and *R. yavapaiensis* were collected under Arizona Game and Fish Permit SP912836 to J. P. Collins; *R. boylii* was collected under California Permit 803036-04 to C. Davidson.

Literature Cited

Berger, L., R. Speare, P. Daszak, D. E. Green, A. A. Cunningham, C. L. Goggin, R. Slocombe, M. A. Ragan, A. D. Hyatt, K. R. McDonald, H. B. Hines, K. R. Lips, G. Marantelli, and H. Parkes. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proc. Natl. Acad. Sci. USA 95:9031–9036.

Bosch, J., I. Martinez-Solano and M. Garcia-Paris. 2001. Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas of central Spain. Biol. Conserv. 97:331–337.

Bradley, G. A., P. C. Rosen, M. J. Sredl, T. R. Jones, and J. E. Longcore. 2002. Chytridiomycosis in native Arizona frogs. J. Wildl. Dis. 38:206–212.

Carey, C., N. Cohen, and L. Rollins-Smith. 1999. Amphibian declines: an immunological perspective. Devel. Comp. Immunol. 23:459–472.

———, D. F. Bradford, J. L. Brunner, J. P. Collins, E. W. Davidson, J. E. Longcore, M. Ouellet, A. P. Pessier, and D. M. Schock. 2003. Biotic factors in amphibian population declines, in press. *In*: Multiple stressors and declining amphibian populations: evaluating cause and effect, G. Linder, D. W. Sparling, and S. K. Krest (eds.). Society of Environmental Toxicology and Chemistry (SETAC), Pensacola, FL.

Collins, J. P., J. Brunner, V. Miera, M. Parris, D. Schock, and A. Storfer. 2003. Ecology and evolution of infectious disease, p. 137–151. *In*: R. Semlitsch (ed.). Amphibian conservation.Smithsonian Institution Press, Washington, DC.

Daszak, P., L. Berger, A. A. Cunningham, A. D. Hyatt, D. E. Green, and R. Speare. 1999. Emerging infectious diseases and amphibian population declines. Emerg. Infect. Dis. 5:735–748.

Davidson, C., H. B. Shaffer, and M. R. Jennings. 2001. Declines of the California red-legged frog: climate, UV-B, habitat and pesticides hypotheses. Ecol. Appl. 11:464–479.

Dawes, C. J. 1971. Biological techniques in electron microscopy. Barnes and Noble, New York.

Fredricks, L. P., and J. R. Dankert. 2000. Antibacterial and hemolytic activity of the skin of the terrestrial salamander, *Plethodon cinereus*. J. Exp. Zool. 287:340–345.

Hopkins, S., and Channing, A. 2002. Chytridiomycosis in Northern and Western Cape frog populations, South Afr. Froglog 54:1–2.

Jancovich, J. K., E. W. Davidson, J. F. Morado, B. L. Jacobs, and J. P. Collins. 1997. Isolation of a lethal virus from the endangered tiger salamander *Ambystoma tigrinum stebbinsi*. Dis. Aquat. Org. 31: 161–167.

Longcore, J. E., A. P. Pessier, and D. K. Nichols. 1999. *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. Mycologia 91: 219–227.

Matutte, B., K. B. Storey, F. C. Knoop, and J. M. Conlon. 2000. Induction of synthesis of an antimicrobial peptide in the skin of the freeze-tolerant frog, *Rana sylvatica*, in response to environmental stimuli. FEBS Lett. 483:135–138.

Nichols, D. K., E. W. Lamirande, A. P. Pessier, and J. E. Longcore. 2001. Experimental transmission of cutaneous chytridiomycosis in dendrobatid frogs. J. Wildl. Dis. 37:1–11.

Pessier, A. P., D. K. Nichols, J. E. Longcore, and M. S. Fuller, 1999. Cutaneous chytridiomycosis in poison dart frogs (*Dendrobates* spp.) and White's tree frogs (*Litoria caerulea*). J. Vet. Diagn. Invest. 11:194–199.

Rollins-Smith, L. A. 2001. Neuroendocrine-immune system interactions in amphibians. Implications for understanding global amphibian declines. Immunol. Res. 23:273–280.

———, C. Carey, J. E. Longcore, J. K. Doersam, A. Boutte, J. E. Bruzgal, and J. M. Conlon. 2002. Activity of antimicrobial skin peptides from ranid frogs against *Batrachochytrium dendrobatidis*, the chytrid fungus associated with global amphibian declines. Devel. Comp. Immunol. 26:471–479.

(EWD, JPC, JB) Department of Biology, Arizona State University, Tempe, Arizona 85287-1501; (MP) Department of Biology, University of Memphis, Memphis, Tennessee 38152-3540; (JEL) Department of Biologi-

CAL SCIENCES, UNIVERSITY OF MAINE, ORONO, MAINE 04469; AND (AP) UNIVERSITY OF ILLINOIS ZOOLOGICAL PATHOLOGY PROGRAM, LOYOLA UNIVERSITY MEDICAL CENTER, MAYWOOD, ILLINOIS 60153. E-mail: (EWD) e.davidson@asu.edu. Send reprint requests to EWD. Submitted: 17 June 2002. Accepted: 26 Feb. 2003. Section editor: S. J. Beaupre.