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PREVALENCE OF *BATRACHOCHYTRIUM DENDROBATIDIS* IN AMERICAN BULLFROG AND SOUTHERN LEOPARD FROG LARVAE FROM WETLANDS ON THE SAVANNAH RIVER SITE, SOUTH CAROLINA

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ABSTRACT: *Batrachochytrium dendrobatidis*, an aquatic fungus, has been linked to recent amphibian population declines. Few surveys have assessed *B. dendrobatidis* infections in areas where the disease is suggested to be less virulent and population declines have not been observed, such as southeastern North America. Although adult *Rana catesbeiana* and *Rana sphenocephala* from the Savannah River Site, South Carolina collected in 1979 and 1982 were identified as having *B. dendrobatidis*, it is unknown whether the fungus is currently present at the site or if susceptibility to infection varies among species or wetlands with different histories of environmental contamination. From 15 May through 15 August 2004, we collected *R. catesbeiana* and *R. sphenocephala* tadpoles from three wetlands with differing contamination histories on the Savannah River Site, South Carolina. We found *B. dendrobatidis* in only one of the wetlands we surveyed. *Batrachochytrium dendrobatidis* infection was identified in 64% of the *R. catesbeiana* tadpoles sampled and histologically assessed ($n=50$) from a wetland contaminated with mercury, copper, and zinc. No *R. sphenocephala* tadpoles from this site ($n=50$) were infected. In combination with a recently published report, our data suggest that *B. dendrobatidis* has been present at the Savannah River Site for over 25 yr but has not caused any apparent population declines. This time period is similar to the known presence of 30 yr of *B. dendrobatidis* in northeastern North America. Our data suggest that *R. sphenocephala* larvae might be resistant to infection, even when occupying the same wetland as the infected *R. catesbeiana*. Our survey did not clarify the effects of environmental contamination on infection severity, but our study stresses the importance of additional field surveys to document how this pathogen is affecting amphibians globally.

Key words: Amphibian decline, *Batrachochytrium dendrobatidis*, chytrid, *Rana*, tadpole, trace element.

INTRODUCTION

The chytrid fungus *Batrachochytrium dendrobatidis* has been linked to amphibian population declines in Australia (Berger et al., 1998), Europe (Bosch et al., 2001), Central America (Berger et al., 1998; Lips et al., 2006), and North America (Green and Sherman, 2001; Muths et al., 2003; Rachowicz et al., 2006). However, few researchers have investigated the prevalence of *B. dendrobatidis* in areas where declines have not been observed. Daszak et al. (2003) suggested that environmental temperature and aspects of host biology could be used to predict susceptibility or resistance of populations to *B. dendrobatidis*. They predicted that amphibians with low fecundity and specialized niches should have elevated susceptibility to *B. dendrobatidis* when environmental temperatures are low, and the opposite effect should be observed in species with high fecundity and a generalized niche when environmental temperatures are high. These predictions were derived from studies characterized by the former description, but studies have not been conducted to evaluate the latter. Surveys are needed for *B. dendrobatidis* in species that are predicted to be resistant to infection to provide insight into the type and extent to which cofactors such as pollution, UV-B

radiation, stress, and compromised immune condition might increase or decrease the susceptibility of a population to infection (Carey et al., 1999; Daszak et al., 2003). Additionally, monitoring populations of unaffected species can allow us to study resistance and identify what factors might contribute to susceptibility.

Amphibian populations at the Savannah River Site (SRS), Aiken, South Carolina have been intensively monitored for the past 40 yr. Both pristine wetlands and wetlands contaminated with various amounts of trace elements occur within this protected 78,000 ha site. Daszak et al. (2005) reported *B. dendrobatidis* infections in three adult frogs (two American Bullfrogs [*Rana catesbeiana*] and one Southern Leopard Frog, [*Rana sphenocephala*]) from 137 museum specimens collected from the SRS between 1940 and 2001. The three infected individuals were collected between 1978 and 1981 and originated from three wetlands (Grassy Pond, Rainbow Bay, and Susan's Swamp). Although the population of *R. sphenocephala* at Rainbow Bay has experienced declines, these declines have been attributed to climatic factors (Daszak et al., 2005). Otherwise, no persistent population declines have been observed in either *R. catesbeiana* or *R. sphenocephala* populations at heavily monitored localities on the SRS (Daszak et al., 2005). The species at this site fit into Daszak's description of resistant species: they exhibit high fecundity, have a generalized niche, and environmental temperatures are high. No other surveys have determined if *B. dendrobatidis* persists in the non-declining populations of amphibians at the SRS or anywhere else in southeastern North America. Because the SRS contains a variety of wetlands ranging from relatively pristine to those receiving contaminants, the possibility exists that if *B. dendrobatidis* still persists on the SRS, infection prevalence might differ among sites with different disturbance histories.

Most studies have focused on *B. dendrobatidis* infection in adults rather than larvae, even though sampling larvae for histologic analysis has been suggested to be the most sensitive and accurate method for identifying *B. dendrobatidis* presence within amphibian populations (Berger et al., 1999, 2000; Daszak et al., 2003). Infections in larvae are limited to the keratinized jaws and tooth rows of the oral disc (Berger et al., 1998). Although *B. dendrobatidis* affects the jaws and tooth rows, it does not cause the mortality documented in adults. In fact, the only study that has found *B. dendrobatidis* to be lethal to larvae is a lab study by Blaustein et al. (2005), which demonstrated higher mortality in infected Western Toads (*Bufo boreas*) compared to uninfected controls. To determine the prevalence of *B. dendrobatidis* infection in tadpoles in an area where frog species might be resistant to lethal effects of infection, in the summer of 2004 we surveyed larval American Bullfrogs and Southern Leopard Frogs in sites with different histories of trace element pollution on the SRS. We wished to determine 1) if *B. dendrobatidis* is currently present; 2) if *B. dendrobatidis* infection prevalence and severity differ within populations exposed to different combinations of trace elements; and 3) if infection prevalence and severity differ between species within sites.

METHODS

Study areas

From 15 May 2004 through 15 August 2004, we collected larval *R. catesbeiana* and *R. sphenocephala* from three wetland areas on the western half of the SRS (Fig. 1) that display different histories of trace element contamination. Site #1 (Ellenton Bay) is a heavily monitored Carolina bay that is commonly used as a reference site by biologists at the Savannah River Ecology Laboratory because it contains no point source of contamination. Carolina bays are isolated, temporary wetlands that receive their water from spring rains. Site #2 is a series of constructed wetlands that receive cooling water, lab drain waste, air stripper effluent,

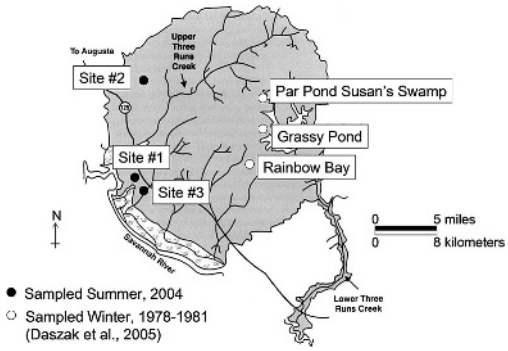

FIGURE 1. Sites surveyed within the Savannah River Site for *Batrachochytrium dendrobatidis* presence within larval *Rana catesbeiana* and *R. sphenocephala* in summer 2004 (black circles) and sites with historically infected adults of these species (white circles).

steam condensate, and storm water from an industrial area on the SRS and has had a history of copper and mercury contamination (Lehman et al., 2002). This drainage consists of a large retention basin that empties into eight 0.5-ha wetland cells designed to sequester any contaminants released from the industrial area. Waters leaving these wetlands empty into Upper Three Runs Creek, a tributary of the nearby Savannah River. Site #3 consists of two settling basins and a drainage swamp that receive sluiced fly and bottom ash from a coal-fired power plant (Rowe et al., 2002). Water exiting the swamp flows into Beaver Dam Creek, another tributary of the Savannah River (Rowe et al., 2002). Site #3 has elevated sediment concentrations of approximately 20 trace elements, several of which (Se, As, Cd) are high enough to be of toxicologic concern (Hopkins et al., 1998; Rowe et al., 2002). Because at least 22 species of amphibians are attracted to the site for foraging, breeding, and overwintering, concern has been raised about how this site and other similar power plant sites in the US could serve as localized population sinks for amphibians (Rowe and Hopkins, 2003).

Areas with history of infection

Daszak et al. (2005) identified three wetlands on the eastern half of the SRS (Fig. 1) that contained infected anurans collected between 1978 and 1981. Susan's Swamp and Grassy Pond are both part of the larger Par Pond area, which drains into the Savannah River via Lower Three Runs Creek. Rainbow Bay is a Carolina bay. These three wetlands are isolated from the three areas surveyed in the current study (Fig. 1).

Tadpole collection, processing, and histologic examination

Approximately 50 tadpoles of each species were collected from sites #1 and #2, but only *R. catesbeiana* were available at site #3. We collected any tadpole with an oral disc (jaws and tooth rows) and did not collect individuals who had begun to lose their oral disc as a normal event during metamorphosis. We did not evaluate presence or absence of oral disc pigment in the field. We transported tadpoles back to the lab, euthanized them with MS-222, and stored them in 10% buffered formalin. Each tadpole's oral disc was removed with a razor and serially dehydrated in ethanol and imbedded in paraffin. Samples were sectioned at 10 μm and stained with hematoxylin and eosin. Sections were viewed with a microscope on 200× magnification to identify *B. dendrobatidis* zoosporangia. Positive identifications of *B. dendrobatidis* zoosporangia (spheres within the epidermis) were verified by researchers experienced in histologic identification of *B. dendrobatidis* (G. Fellers, D. Green, D. Mendez, L. Rachowicz, R. Speare, and V. Vredenburg). If any of the sections from an individual contained *B. dendrobatidis* zoosporangia, we considered the individual infected; if no sections contained zoosporangia, we considered that individual uninfected.

Of the sections that contained zoosporangia, those that were sagittally sectioned ($n=14$) were qualitatively assessed for location of infection within the oral disc and the severity of infection. Infections were grouped into six locations: anterior teeth, anterior jaw, anterior buccal epithelium, posterior buccal epithelium, posterior jaw, and posterior teeth. Infected localities within the oral disc were given a severity score based on qualitative characteristics of the infection. A score of one was assigned to areas in which infection was light, meaning that between 1 and 10 zoosporangia were spaced within the superficial epidermis. If zoosporangia were clustered along the superficial epidermis, two to three zoosporangia deep into the epidermis, and contained between 20 and 50 zoosporangia, we assigned a score of two. A score of three was given to areas with clusters of zoosporangia numbering more than 50.

Trace element preparation and analysis

Additional tadpoles ($n=8$) were collected from each site to determine trace element

concentrations accumulated by individuals. *Rana catesbeiana* tadpoles were collected from all three sites, but *R. sphenocephala* tadpoles were only collected from site #2. Tadpoles were housed in the lab for 48 hr after collection to void excessive sediments from their guts (Burger and Snodgrass, 1998). Tadpoles were then snap-frozen, lyophilized, and homogenized prior to trace element analysis. Concentrations of 13 trace elements (As, Cd, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Se, Sr, V, and Zn) were determined using an inductively coupled plasma mass spectrometer (Elan DRC plus, Perkin Elmer, Norwalk, Connecticut, USA). These trace elements were chosen because they occur in relatively high concentrations in sediment, water, and biota at Site #2 and/or #3 (Hopkins et al., 1998, 2000; Rowe et al., 1998; Lehman et al., 2002). A detailed description of digestion (EPA method 3052) and trace element analysis (EPA method 6020a) procedures can be found in Hopkins et al. (2004). Recovery of trace elements from the standard reference material TORT-2 (Lobster hepatopancreas; NRCC, Ottawa, Ontario Canada) ranged from 76% (Ni) to 102% (Se) of the certified value ($n=12$). Analytical spike recovery ranged from 94% in V to 124% in Sr. The mean relative percent difference (RPD) between replicate dilutions and analyses of the digestates ranged from 0.45% in Pb to 4.07% in Hg. Method detection limits (MDLs) in dry samples depended on sample size but ranged from 0.030 μg/g dry mass in Hg to 132 μg/g dry mass in Zn.

Statistical analyses

Percentages of infected individuals were compared among and within sites via Chi square contingency tests. To determine the accuracy of these percentages, we calculated 95% binomial confidence intervals (Conover, 1980). We used a series of analyses of variance (ANOVAs) of principal component analysis (PCA) scores for each *R. catesbeiana* tadpole, to investigate differences in tadpole element concentrations among sites. To investigate differences between *R. catesbeiana* and *R. sphenocephala* element concentrations from site #2, we used a multivariate ANOVA (MANOVA). Scheffe's range tests were conducted for all a posteriori comparisons. All trace element concentrations were $\log_{10}$ transformed to better fit the assumptions of ANOVA and MANOVA. We used StatView for Windows (SAS Institute, version 5.0.1) for all statistical analyses.

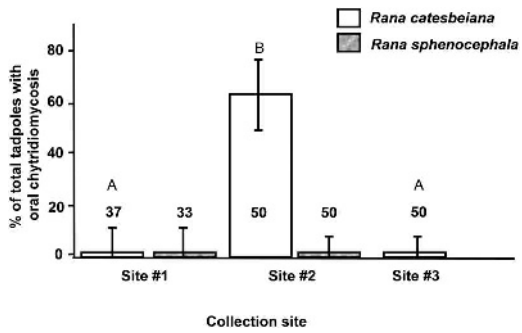

FIGURE 2. Prevalence of *Batrachochytrium dendrobatidis* within *Rana catesbeiana* and *R. sphenocephala* tadpoles from three wetlands from the Savannah River Site, Barnwell County, South Carolina. Error bars indicate 95% binomial confidence intervals. Percentages labeled with a different letter denote a significant difference (Chi square contingency test, $P<0.01$) among sites. The percent of *R. catesbeiana* and *R. sphenocephala* within site #2 were also significantly different (Chi square contingency test, $P<0.01$).

RESULTS

Tadpoles collected and infection prevalence

Rana catesbeiana tadpoles were collected at all three sites (Fig. 2). *B. dendrobatidis* infections were identified in *R. catesbeiana* from the site with a history of Cu and Hg contamination (site #2). No *R. catesbeiana* tadpoles from the reference site (site #1) or the coal power plant settling basin swamp (site #3) were infected. The percentage of infected *R. catesbeiana* from site #2 was high (64%) and significantly different from sites #1 and 3 (Fig. 2; $\chi^2=72.6$, $P<0.01$). *Rana sphenocephala* were collected only from sites #1 and 2. None of the *R. sphenocephala* sampled from either of the two sites were infected with *B. dendrobatidis*. Infection prevalence between the two species at site #2 were significantly different ($\chi^2=47.1$, $P<0.01$). Both species from site #2 were caught within the same minnow traps suggesting that they are syntopic within the wetland. Although developmental stages were not recorded at collection, most *R. catesbeiana* tadpoles from site #2 were in the early stages of hind limb development and most *R.*

TABLE 1. Infection location and severity within the oral disc of *Rana catesbeiana* collected from a site with a history of Cu and Hg contamination (site #2) on the Savannah River Site, South Carolina. The number of infected oral discs (# infected) of the number of oral discs that retained the specified feature of the oral disc (*n*) is reported as a percentage (% infected). These locations were given a severity score (see methods for description of severity scores).

Location	Number infected	*n*	% infected	Severity
Anterior				
Tooth rows	2	8	20%	1
Jaw	4	13	31%	2
Buccal epithelium	9	13	69%	2–3
Posterior				
Tooth rows	7	10	70%	1
Jaw	2	14	14%	2
Buccal epithelium	11	14	79%	2–3

sphenocephala were in the late stages of hind limb development.

Although our infection prevalence describes the percentage of infected individuals within the animals sampled, binomial confidence intervals predict the actual prevalence within the population. Our binomial confidence intervals varied with site and species (Fig. 2). *Rana sphenocephala* had confidence intervals of 0–12% at site #1 and 0–7.5% at site #2. Thus, although we found no infected individuals for this species at either site, the actual percentage of infected individuals within these populations has a 95% probability of ranging from 0–12% at site #1 and 0–7.5% at site #2. Confidence intervals for *R. catesbeiana* were 0–12% at site #1, 49–77.5% at site #2, and 0–7.5% at site #3.

Infection description, location, and severity

Qualitative histologic analyses suggested that infections tend to occur in specific locations within the oral disc (Table 1). Not every slide retained all six parts of the oral disc after histologic preparation, so sample sizes vary among infection locations. For example, anterior tooth rows were retained in eight of the 14 sagittally sectioned slides for each individual. We found infections of the buccal epithelium more frequently than infections of the jaw or tooth rows. All of the infected tadpoles had zoosporangia in either the anterior and/or posterior buccal epithelium. The anterior buccal epithelium was infected in 69.2% of the oral discs that retained that feature, and when present, 78.6% of posterior epithelia were infected. These cases were designated a severity score of two (Fig. 3). Tooth rows were infected in 20% (anterior) and 70% (posterior) of the oral discs that retained tooth rows after histologic preparation. These infections were given a severity rank of one. Infections on the anterior and posterior jaw sheaths were noted in 30.8% and 14.3% of the oral discs examined. These infections tended to be similar to the infections of the buccal epithelium, so they were given a score of two. Although previous researchers (Fellers et al., 2001) noted deformation (rounding) of the cutting edge of the jaws, we did not observe this phenomenon.

Trace element accumulation

Principal component analysis of *R. catesbeiana* trace element accumulation identified three axes that collectively explained 97% of the variance in the data. The first PCA axis (Factor I) represents the strong positive correlation ($\geq$0.70) among As, Fe, Mo, Ni, and V (Table 2). Principal component analysis axis II represents the strong positive correlation among Cu, Hg, and Zn. Principal component analysis axis III represents the positive correlation between Se and Sr, and their negative correlation with Pb and Mn. Cadmium was moderately correlated (0.50–0.70) with all three axes. Selenium and Sr were moderately correlated with axis I and As and Mo were moderately correlated with axis III. Body size was excluded from our analysis after an initial PCA and ANCOVA, using body size as a covariate, found it did not significantly affect the results.

Mean PCA scores for *R. catesbeiana*

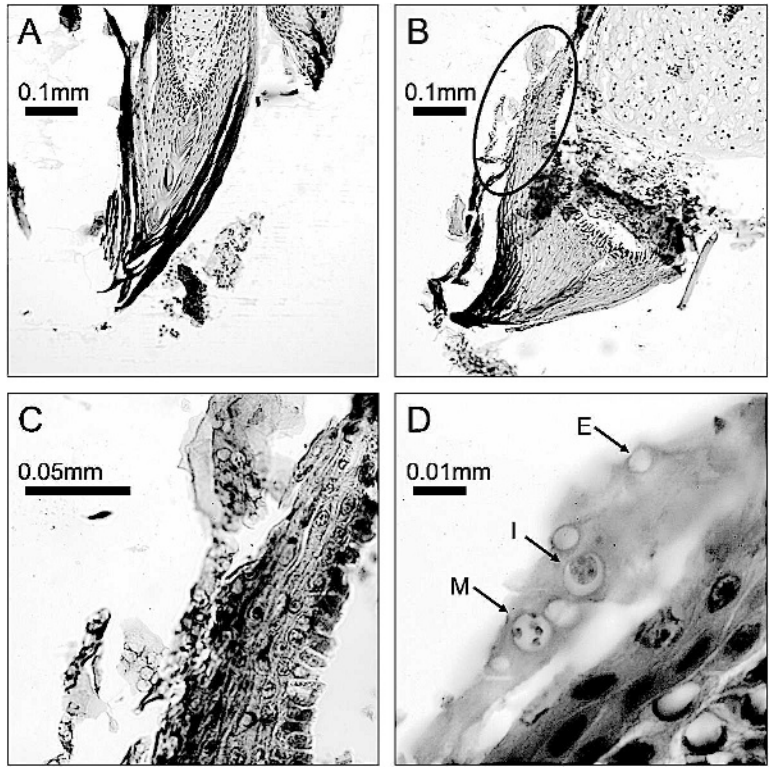

FIGURE 3. Sagital sections of keratinized jaw sheaths from *Rana catesbeiana* tadpoles collected summer 2004 from sites #1 (A) and #2 (B–D), Savannah River Site, Barnwell County, South Carolina. (A) Normal jaw sheath lacking oral chytridiomycosis. (B) Jaw sheath (25× magnification) with oral chytridiomycosis superficially located on the distal portion of the jaw sheath (circled). (C) 100× magnification. (D) 250× magnification: an empty zoosporangium (E) and zoosporangia containing immature zoospores (I) and mature zoospores (M) can be seen.

trace element accumulation differed significantly among sites for all three axes (ANOVA, $P<0.05$; Fig. 4). Site #3 was significantly different than sites #1 and 2 for PCA axes I and III, suggesting that tissue concentrations of As, Fe, Mo, Ni, Se, Sr, and V were significantly higher in *R. catesbeiana* from site #3 (Scheffe's range test, $P<0.05$). Mean PCA scores for axis II were all significantly different among sites; tissue concentrations of Cu, Hg, and Zn were highest at site #2, intermediate at site #3, and lowest at site #1. The strong negative correlation of Mn and Pb on axis III suggests that accumulation of these metals was significantly greater at sites #1 and 2.

Trace element concentrations differed significantly between species at site #2 (MANOVA; $P<0.01$; Table 3). Concentrations of As, Cd, Fe, Hg, Mo, Ni, Pb, and V were significantly higher in *R. sphenocephala* and Se was significantly higher in *R. catesbeiana* (Scheffe's range test, $P<0.05$).

DISCUSSION

Our finding of *B. dendrobatidis* within amphibians on the SRS suggests that this fungus has persisted at this location for at least 25 yr. This is the longest known time period that *B. dendrobatidis* has been documented to be present at one geographical area within southeastern North America; however, Ouellet et al. (2005) found that *B. dendrobatidis* has been present at the Mont Saint-Hilaire Biosphere Reserve, Quebec, Canada since the

TABLE 2. Loadings of trace elements on the first three principal component axes resulting from a principal component analysis of trace element concentrations in tissues of *Rana catesbeiana* larvae from wetlands on the Savannah River Site, South Carolina. Only loadings ≥0.50 and ≤−0.50 are shown for clarity.

	Factor		
Variable	I	II	III
As	0.756		0.640
Cd	0.608	0.524	0.586
Cu		0.977	
Fe	0.923		
Hg		0.985	
Mn			−0.971
Mo	0.774		0.619
Ni	0.910		
Pb			−0.895
Se	0.641		0.760
Sr	0.539		0.795
V	0.892		
Zn		0.868	
% of variance explained	61.2	22.3	13.5

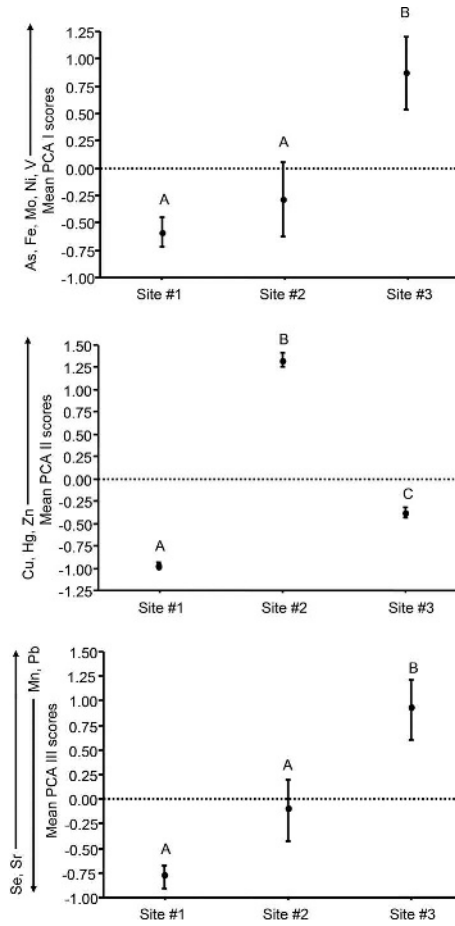

FIGURE 4. Mean *Rana catesbeiana* trace element principal component analysis scores among sites for each principal component analysis factor. Error bars indicate ±1 standard error of the mean. Sites were statistically different (ANOVA, $P<0.05$) for all three factors. Means labeled with a different letter denote a significant difference (Sheffe's post hoc comparison, $P<0.05$). Trace elements on the Y-axis identify which elements are correlated with each axis. Arrows identify whether the correlation is positive (↑) or negative (↓). Figure adapted from Snodgrass et al. (2003).

1960s. Although population declines have occurred in at least four species (*Ambystoma talpoideum, A. tigrinum, Pseudacris ornata*, and *R. sphenocephala*) native to the SRS and in Western Chorus Frogs (*Pseudacris triseriata*) native to Quebec, these declines are more likely associated with droughts (Daszak et al., 2005) and habitat loss (Ouellet et al., 2005). These findings suggest that *B. dendrobatidis* is either endemic to eastern North America or was introduced before 1960. One possible limiting factor for *B. dendrobatidis* at the SRS is high temperatures in the summer. *Batrachochytrium dendrobatidis* does not grow at temperatures above 28 C in vitro (Piotrowski et al., 2004); it is not unusual for average water temperature to exceed this temperature in shallow water wetlands on the SRS in summer months (Schalles et al., 1989). Surveys of *R. catesbeiana* collected in winter or from higher elevations are needed to determine the extent to which temperature affects infection severity in this species.

Rana catesbeiana tadpoles from site #2 had a relatively high infection prevalence (64%, $n=50$) similar to that found in declining Mountain Yellow-legged Frog (*Rana muscosa*) tadpoles (66.7%, $n=24$; Fellers et al., 2001). However, as a species, *R. catesbeiana* is not declining and adults are resistant to severe infections (Daszak et al., 2004). The high percentage of infected *R. catesbeiana* tadpoles within apparently stable populations of this spe-

TABLE 3. MANOVA results comparing trace element accumulation in *Rana catesbeiana* and *Rana sphenocephala* tadpoles collected from site #2, Savannah River Site, South Carolina. Overall, the species were significantly different, (MANOVA, $P=0.0030$). P values marked with an asterisk denote significant differences between the species if $P \leq 0.05$ (Sheffe's post hoc comparison).

	R. catesbeiana			*R. sphenocephala*			
Element	Mean	±	SD[a]	Mean	±	SD[a]	*P*
As	1.20	±	0.29	1.91	±	0.87	0.0253*
Cd	1.20	±	0.24	1.82	±	0.14	0.0001*
Cu	249.96	±	78.27	300.17	±	55.61	0.1261
Fe	2,553.32	±	1,062.23	7,665.98	±	2,709.05	<0.0001*
Hg	0.28	±	0.06	0.67	±	0.25	0.0002*
Mn	164.76	±	61.62	251.69	±	153.13	0.1079
Mo	0.33	±	0.12	0.75	±	0.21	0.0002*
Ni	4.17	±	0.96	6.42	±	1.47	0.0025*
Pb	6.36	±	2.83	17.20	±	7.00	0.0006*
Se	3.38	±	0.90	2.35	±	0.58	0.0094*
Sr	29.14	±	14.60	23.60	±	4.75	0.4194
V	4.53	±	1.95	9.64	±	2.66	0.0008*
Zn	358.70	±	92.63	1,287.49	±	414.50	<0.0001*

[a] SD = Standard deviation.

cies on the SRS suggests that infection prevalence alone does not predict how a species will ultimately be affected by *B. dendrobatidis*. More detailed descriptions of tadpole infections are needed to make comparisons among species to determine the severity and ultimate outcome of infections. For example, Fellers et al. (2001) depicted one *R. muscosa* oral disc infected throughout the entire jaw sheath. The infection was several (ca. 8) zoosporangia deep, and caused marked rounding of the jaw sheath's cutting edge. In contrast, infections in our *R. catesbeiana* tadpoles were limited to specific areas, such as tooth rows and buccal epithelia, between 1 and 3 zoosporangia deep, and we found no rounding of the cutting edge. Unfortunately, detailed descriptions of *B. dendrobatidis* infections in tadpoles are rare in the literature. To facilitate comparisons among studies, future surveys should include identification of the location of infections and quantification of the number of zoosporangia within these locations of the oral disc.

Infection prevalence was drastically different between species within site #2, suggesting that *R. sphenocephala* tadpoles might be resistant to *B. dendrobatidis* infection. Daszak et al. (2004) suggested that *R. catesbeiana* adults become infected when inoculated with *B. dendrobatidis*, but do not develop serious lesions. Our study is the first to report resistance to infection by larvae of a species that occupy the same water as infected larvae of a second species. No zoosporangia were identified within any of the 50 *R. sphenocephala* tadpoles collected from site #2, which contained a high percentage of infected *R. catesbeiana*. In a related laboratory study (J.D.P., V. A. Peterson, and M.T.M., unpubl. data), we inoculated 48 *R. sphenocephala* larvae with a concentration consistent with other infection studies (ca. 3,000 zoospores/ml in 8 l of water for 2 wk; Blaustein et al., 2005) and found no histologic evidence of infection. Parris and Baud (2004) infected larval *R. sphenocephala* in mesocosms with approximately 7,000 zoospores/ml, which later resulted in recently metamorphosed frogs being infected. Because infection was assessed only when metamorphosis was completed, however, it is unclear whether the tadpoles' oral discs were infected or whether the fungus remained alive in the mesocosm and infected animals after they

began metamorphosis. We suggest that future studies should focus on exposing *R. sphenocephala* and other larval anurans to various concentrations of zoospores to more fully understand the range and extent of infections in tadpole oral discs.

Our findings were inconclusive in regard to whether environmental factors, such as trace elements, might be associated with the current presence of *B. dendrobatidis* on the SRS. The only site that we surveyed that contained infected tadpoles was site #2, which was historically contaminated with copper and mercury. *Rana catesbeiana* tadpoles from site #2 accumulated significantly higher concentrations of Cu, Hg, and Zn compared to those from the reference and the coal power plant settling basin wetlands. When trace element concentrations were compared between the two *Rana* species at site #2, the only metal that was significantly elevated in infected *R. catesbeiana* was Se. In fact, the uninfected *R. sphenocephala* from site #2 accumulated significantly higher concentrations of As, Cd, Fe, Hg, Mo, Ni, Pb, and V than *R. catesbeiana*. Burger and Snodgrass (1998) also found Cd, Mn, and Pb concentrations to be higher in *R. sphenocephala* than *R. catesbeiana* and suggested that *R. sphenocephala* might absorb or bioaccumulate trace elements to a greater extent than *R. catesbeiana*. Although trace element accumulation might increase susceptibility to infection, trace elements might also protect individuals from infection (Lawrence, 1981) or even produce conditions inhospitable for certain pathogens. Unfortunately, because of the correlative nature of this field survey, we cannot say with certainty whether the presence of certain combinations of trace elements influenced *B. dendrobatidis* infection; broader surveys that include more sites with a diverse array of environmental conditions are needed.

In conclusion, our findings support the predictions made by Daszak et al. (2004). We collected amphibians with high fecundity and generalized niches during a season when environmental temperatures were high and found these tadpoles to be relatively unaffected by *B. dendrobatidis* infection. No unexplainable population declines have occurred at the SRS. *Rana catesbeiana* larvae retained relatively light infections when compared to declining *Rana mucosa* from the Sierra Nevada Mountains in California, and *R. sphenocephala* appeared to be resistant to infection under the conditions we surveyed. Species-specific susceptibility to infection makes determining the effects of *B. dendrobatidis* complex and illustrates the necessity to examine many amphibian species across diverse environments.

As wildlife species are exposed to increasing numbers of diseases, the need for studies on how diseases persist and move among populations and species becomes urgent. Movement of *B. dendrobatidis* among amphibian populations has been implicated in multiple extinctions, many of which have occurred at alarming rates in relatively pristine habitats (Lips et al., 2006; Pounds et al., 2006). Clearly, additional studies on this devastating pathogen are needed. Specifically, field surveys need to be conducted in areas where *B. dendrobatidis* occurs, especially in sites where identified cofactors such as contaminants are also present. Future surveys should describe severity of infection in tadpoles and how severity is affected by natural and anthropogenic environmental factors. Surveys could provide a foundation for laboratory experiments on the complicated interplay between environmental factors and *B. dendrobatidis* infection that are needed to understanding the global effect of this pathogen and what environmental conditions might influence its threat to amphibians.

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