

AMPHIBIAN POPULATION DECLINES AT SAVANNAH RIVER SITE ARE LINKED TO CLIMATE, NOT CHYTRIDIOMYCOSIS

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Abstract. Amphibian populations at the Savannah River Site (SRS), South Carolina, USA, have been censused consistently for 35 years, and this provides a time series to examine the causes of population fluctuations. We examined archived museum specimens of 15 anuran species collected at wetlands on the SRS for the presence of the causative agent (*Batrachochytrium dendrobatidis*) of chytridiomycosis, an emerging disease associated with population declines elsewhere. Infections were present in three out of 137 (2.18%) individuals; the pathogen was detected in two *Rana catesbeiana* and a single *Rana sphenocephala*, all collected between 1978 and 1981. Lesions were not consistent with the later stages of fatal chytridiomycosis. Analysis of population trajectories of nine amphibian species over 26 years at SRS showed that four species declined significantly over this period, including *R. sphenocephala*. However, we demonstrate that these declines are more likely caused by an increase in the number of years with insufficient rainfall and a shortened hydroperiod at the breeding site than by chytrid epidemics. This pattern appears to be linked to a drying trend at SRS through the 1990s, although it is unclear whether this was caused by climate change. This study demonstrates that the presence of *B. dendrobatidis* in amphibian communities where some species are declining does not always implicate chytrids as a cause of the decline. Like many other emerging pathogens, the outcome of infection can vary among individuals and populations, depending on life history traits, environmental conditions, and virulence factors of the pathogen. Our report also demonstrates the usefulness of archived museum specimens and long-term population monitoring in studying the host–parasite ecology of emerging diseases.

Key words: amphibian population declines; *Batrachochytrium dendrobatidis*; chytridiomycosis; climate change; disease ecology; host–parasite ecology; hydroperiod; Savannah River.

INTRODUCTION

Chytridiomycosis is an emerging fungal disease of amphibians that was first reported in 1998 associated with mass mortality and population declines of montane rainforest amphibians from Panama and Australia (Mahony 1996, Berger et al. 1998, Lips 1998, 1999). The disease is caused by a chytrid fungus, *Batrachochytrium dendrobatidis*, that infects keratinaceous epidermal cells of adult amphibians and keratinized mouthparts of larvae (Berger et al. 1998, Longcore et al. 1999). The pathogen has since been reported from North and South America, Europe, New Zealand, and Africa as the cause of amphibian mass mortality and, in many cases, population declines (Ron and Merino 2000, Bosch et al. 2001, Waldman et al. 2001, Muths et al. 2003, Hanselmann et al. 2004, Weldon et al. 2004). However, in some studies *B. dendrobatidis* has been reported in species that have not undergone declines, even though they are sympatric with declining

species (Berger et al. 1998). This suggests that the impact of *B. dendrobatidis* on amphibian populations may be affected by host traits such as fecundity and niche specialization (Williams and Hero 1998, Daszak et al. 1999).

The aim of the current study was to test the hypothesis that presence of *B. dendrobatidis* in amphibian populations does not always cause long-term population declines. To do this, we examined archived amphibians from the Savannah River Site (SRS), an 803-km² area with amphibian populations for which long-term survey data exist (Gibbons and Bennett 1974, Pechmann et al. 1991, Semlitsch et al. 1996), for the presence of *B. dendrobatidis*. Previous authors have suggested that climate change (Rohr and Madison 2003) or an interaction between climate change and disease (Pounds et al. 1999) may be causally involved in amphibian declines. We related trends in amphibian breeding population size and recruitment of juveniles to temporal variation in rainfall and wetland hydrology to assess whether the presence of *B. dendrobatidis* or the environmental variables provided a better explanation of species declines.

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TABLE 1. Amphibian specimens collected at the Savannah River Site (South Carolina, USA) and surveyed histologically for chytridiomycosis in the current study.

Species	Date unknown	1940–1970	1970–2001	Total positive
<i>Acris crepitans</i>	0	1	0	0
<i>Acris gryllus</i>	0	10	0	0
<i>Bufo quercicus</i>	0	1	0	0
<i>Bufo terrestris</i>	0	18	0	0
<i>Gastrophryne carolinensis</i>	0	3	0	0
<i>Hyla chrysocelis</i>	0	2	0	0
<i>Hyla cinerea</i>	0	2	0	0
<i>Hyla crucifer</i>	0	11	1	0
<i>Hyla gratiosa</i>	0	0	6	0
<i>Rana catesbeiana</i>	0	4	9	2
<i>Rana clamitans</i>	0	4	2	0
<i>Rana grylio</i>	0	2	0	0
<i>Rana sphenoccephala</i>	0	28	15	1
<i>Rana virgatipes</i>	0	0	1	0
<i>Scaphiopus holbrookii</i>	1	7	9	0

MATERIALS AND METHODS

Amphibians were collected at the SRS in Aiken and Barnwell Counties, South Carolina, and deposited in the Georgia Museum of Natural History (UGMNH) between 1940 and 2001 (Table 1). Chytridiomycosis has been reported predominantly from bufonids and ranids in the United States during winter and spring die-offs (Daszak et al. 2003). Therefore, to test for the presence of chytridiomycosis within the stable amphibian populations at SRS, we optimized the chance of finding positive samples by surveying specimens of ranid, hylid, microhylid, pelobatid, and bufonid species collected during the coolest months of the year (September to April). Tissue samples were selected to maximize the chance of finding chytridiomycosis, which primarily affects the ventrum (groin and drink patch) and digits. Three toes, the webbing between them, groin skin, and ventral thigh skin from each frog were collected and processed for histology. Most individuals deposited in the UGMNH had originally been fixed in neutral-buffered 10% formalin, and then placed in 70% alcohol or equivalent for long-term storage. Tissue samples for our study were placed in formalin for 24 h, washed in 0.1 mol/L phosphate buffer overnight, dehydrated in a graded series of increasingly concentrated ethanol solutions, embedded in paraffin wax, sectioned at 4- μ m thickness, stained with haematoxylin and eosin, and examined on a light microscope for evidence of *Batrachochytrium dendrobatidis*. Toes and skin were processed together and sectioned to give slides with longitudinal sections of toes and transverse sections of skin visible. Twenty-five low-power fields of view were examined for each of two slides per frog.

Population counts of nine amphibian species were conducted at Rainbow Bay, a 1-ha seasonal wetland on the Savannah River Site, from 1979 to 2004, as described previously (Pechmann et al. 1991). Briefly, animals were captured in 40-L pitfall traps along a ter-

restrial drift fence that surrounds the wetland. Traps were checked daily, and captured animals were marked, sexed, recorded, and released on the opposite side of the drift fence. These data allowed us to determine yearly totals for each species of the number of breeding females (i.e., breeding population size) and the number of offspring that metamorphosed successfully from the breeding site (i.e., the number of juveniles produced). However, bullfrogs, *Rana catesbeiana*, rarely breed in Rainbow Bay (due to its ephemeral nature) and adults are able to jump over the drift fence. As a result, the population counts that we analyzed for *R. catesbeiana* are simply the total number of individuals trapped, which represent primarily immature individuals that are dispersing from nearby wetlands as well as juveniles that were produced at Rainbow Bay in 1979, 1980, 1984, and 1991. We examined population trends in *R. catesbeiana*, despite these challenges, because bullfrogs were one of the species shown to be infected with *B. dendrobatidis* (see RESULTS). It should also be noted that the drift fence method captures only those adults that are migrating to the wetland to breed, and does not assess the entire population (Semlitsch et al. 1996). The number of breeding females trapped in a given year depends not only on survival and recruitment in prior years, but also on the suitability of environmental conditions for migrations to the breeding site; drier years may reduce the likelihood that an individual will migrate.

Population analyses

Population declines were assessed by regressing the log-transformed number of females (0.5 was added to zero values) caught in pitfall traps in year t , N_t , against time (a simple index of years since 1979)

$$N_t = c_0 + c_2 \times \text{year} + e_t$$

where c_0 and c_2 are fitted coefficients and e_t is an error term. Since the number of females trapped in successive years are not independent of one another, we used parametric bootstrapping (sensu Dennis and Taper 1994) to determine the significance of the coefficient c_2 . To do so, we fit the log-transformed population data to an autoregressive (AR1) model

$$N_t = c_0 + c_1 \times N_{t-1} + e_t.$$

We then used the coefficients c_0 and c_1 and the estimated variance of e_t (which were normally distributed for all species; Kolmogorov-Smirnoff test for normality; all $P > 0.15$) to generate time series of equal length to the simulated data. We regressed these data against the year index to quantify the likelihood of seeing a similar increase or decrease in abundance over time. We used 10 000 simulations and generated 95% confidence intervals and approximate P values for the null hypothesis that $c_2 = 0$.

To understand the observed declines of some species, we examined how the number of juveniles produced

TABLE 2. Regression coefficients of amphibian populations regressed against time, P values estimated using parametric bootstrapping, minimum hydroperiods for production of normal juveniles, the geometric mean number of breeding females trapped in years with and without sufficient hydroperiods ("wet" and "dry," respectively), and t test comparison of log-transformed data.

Species	c_0	c_2 (time)	P (time)	Minimum hydroperiod (d)	No. females trapped		Comparison (df = 22)	
					Dry	Wet	t	P
<i>Ambystoma talpoideum</i>	6.6	-0.070	0.030	142	131.7	579.0	3.62	0.0015
<i>Ambystoma opacum</i>	1.9	0.224	0.005	54	524.7	97.5	-3.01	0.0064
<i>Ambystoma tigrinum</i>	3.7	-0.164	0.016	154	1.7	16.5	4.43	0.0002
<i>Scaphiopus holbrookii</i>	1.6	0.037	0.435	56	1.7	13.8	2.4	0.043
<i>Bufo terrestris</i>	3.6	0.012	0.625	56	18.2	70.2	4.66	0.0001
<i>Pseudacris ornata</i>	5.9	-0.242	0.024	85	2.8	26.6	2.66	0.019
<i>Gastrophryne carolinensis</i>	5.0	-0.020	0.358	30	106.1	128.8	0.77	0.45
<i>Rana sphenoccephala</i>	4.0	-0.149	0.034	110	2.9	13.6	2.38	0.032
<i>Rana catesbeiana</i>	2.5	-0.020	0.660	NA	NA	NA		

Note: NA, not applicable; see *Methods*.

each year varied in relation to pond hydroperiod (i.e., the number of days that the pond had standing water) and the timing of pond drying. Successful production of juveniles required that the pond have standing water for long enough for normal development and metamorphosis at the appropriate period in the species breeding phenology. We estimated the minimum hydroperiod for successful breeding for each species by examining the number and body size of juveniles trapped each year (Table 2; D. E. Scott, unpublished data). We then divided years of trapping data based on whether there was a sufficient hydroperiod for successful breeding and larval development for each species. This produced estimates of abundance during what we term wet "development" years and drought years (years with insufficient hydroperiod for larval development to be completed).

RESULTS

We examined a total of 137 anurans histologically (Table 1). The causative agent of chytridiomycosis, *Batrachochytrium dendrobatidis*, was identified in histological sections from two bullfrogs (*Rana catesbeiana*) collected in 1978 and 1980 (UGMNH catalog number 44741, collected on 1 December 1978 at Par Pond Susan's Swamp, Barnwell County by R. D. Semlitsch and UGMNH number 44743, collected on 30 January 1980 at Grassy Pond by J. P. Caldwell) and a single *Rana sphenoccephala* collected in 1981 (UGMNH number 44921, collected on 20 January 1981 at Rainbow Bay by J. W. Gibbons and G. W. Esch). Identification was based on the presence of septate sporangia in epidermal keratinaceous cells (Appendix) and the ultrastructural identification of flagellated zoospores within such sporangia (data not shown). Lesions were focal (up to 100 μ m in cross-sectional diameter), with hyperplasia of the keratinaceous cells and hyperkeratosis (Appendix).

We examined population fluctuations of *Rana sphenoccephala*, *R. catesbeiana*, and seven other amphibians at Rainbow Bay from 1978 to 2004. Fig. 1 shows the

number of breeding females and the number of juveniles caught in pitfall traps each year and years where the species-specific developmental hydroperiod was shorter than the minimum time necessary to complete larval development. Regression analysis of these population trends is given in Table 2. Four species, *Ambystoma talpoideum*, *A. tigrinum*, *Pseudacris ornata*, and *Rana sphenoccephala* (which was positive for *B. dendrobatidis* in 1981) showed significant declines, whereas one species, *Ambystoma opacum*, showed a highly significant increase in abundance over the period 1978–2004 (Table 2). Four species, *Scaphiopus holbrookii*, *Bufo terrestris*, *Gastrophryne carolinensis*, and *R. catesbeiana* (which was positive for *B. dendrobatidis* at Par Pond in 1978 and 1980) showed no significant trends in population abundance over time.

The frequency of insufficient hydroperiod years has increased markedly over the past 26 years for the four declining species (Fig. 1), and the number of breeding females that were trapped in these dry years was significantly lower (Table 2). In contrast, two of the species that were not significantly declining (*G. carolinensis* and *A. opacum*) are present at similar or higher numbers in years with insufficient hydroperiods, and the remaining two species (*B. terrestris* and *S. holbrookii*) require very short hydroperiods (Table 2). A correlation of the time coefficient c_2 against the log transformed ratio of females trapped in years with and without sufficient hydroperiod is significantly positive ($r = 0.81$, $n = 8$, $P = 0.016$). In addition, two of the four species that are declining (*A. talpoideum* and *A. tigrinum*) require longer hydroperiods (Table 2), resulting in numerous years with insufficient length hydroperiods for successful reproduction (Fig. 1). Finally, some species whose populations do not appear to be declining have 10 or more consecutive years without successful reproduction.

DISCUSSION

Chytridiomycosis is an emerging infectious disease responsible for mass mortality of a range of wild am-

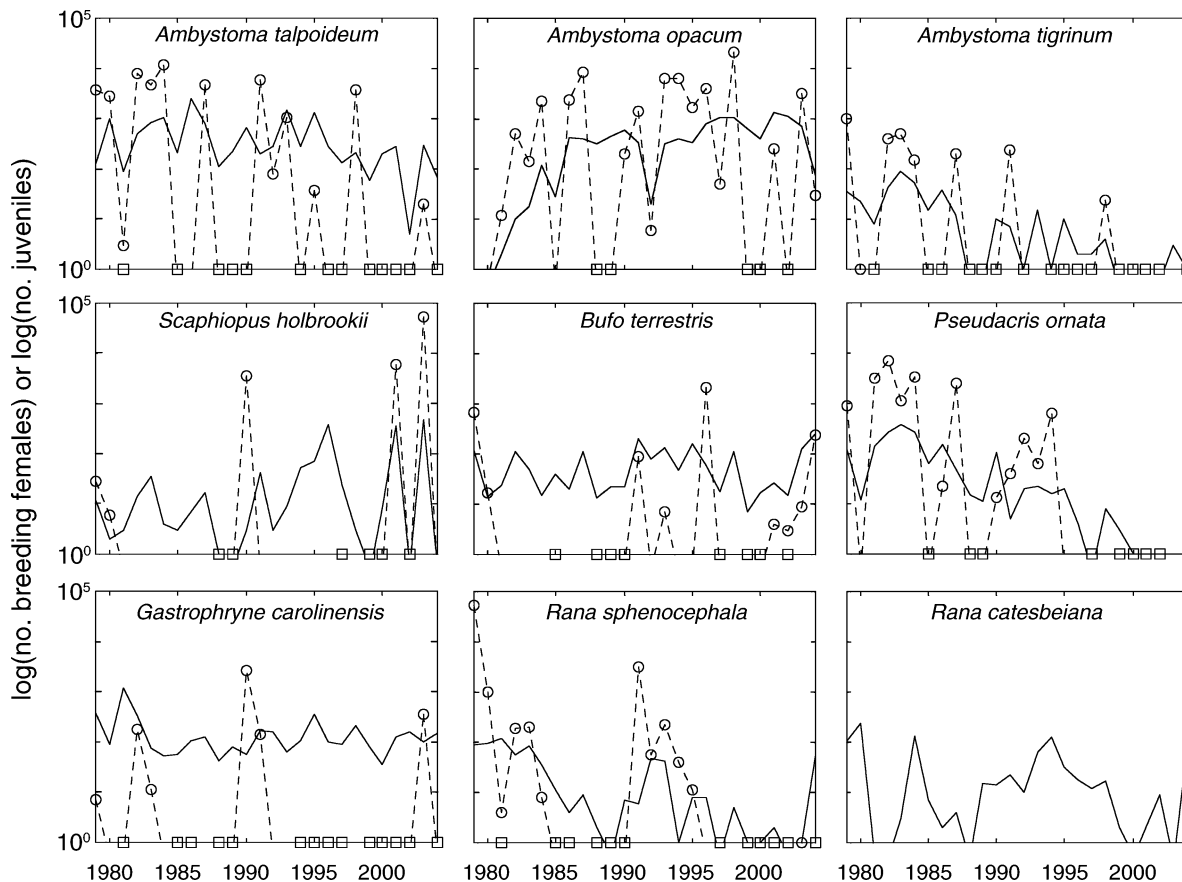


FIG. 1. Population censuses for nine species of amphibians at Rainbow Bay, Savannah River Site, 1979–2004. For *R. catesbeiana*, the solid line represents the number of juvenile individuals trapped that year (see *Methods*). For other species, plots show the number of breeding females (solid line) and juveniles (dashed line) caught in pitfall traps, and years with insufficient hydroperiod (squares) for successful development of larvae for that species (see *Methods* and Table 1).

phibian species in tropical (Berger et al. 1998) and temperate zones (Muths et al. 2003) and of captive animals (Pessier et al. 1999). It infects frogs, toads, and salamanders (including ambystomatids) and has been implicated in local population extinctions, sustained population declines, and even species extinctions (Berger et al. 1998, Daszak et al. 1999, 2003, Davidson et al. 2003). Our study demonstrates that the causative agent of chytridiomycosis, *Batrachochytrium dendrobatidis*, was present in amphibian populations at the Savannah River Site (SRS), South Carolina during the period 1978–1981. Although we examined 104 amphibian specimens deposited in the UGMNH prior to 1978 and found no evidence of *B. dendrobatidis*, we cannot exclude the possibility that the pathogen was present at SRS at low (<7%) prevalence.

At Rainbow Bay, SRS, we found evidence of population declines in four species over the period 1978–2004, including *R. sphenocephala*, in which *B. dendrobatidis* was present in 1981. However, our analyses of population trends suggest that these declines are attributable to an increase in the frequency of droughts at the Savannah River Site between 1978 and 2004

(Pechmann et al. 1991, Semlitsch et al. 1996). During these droughts, the length of time that Rainbow Bay had standing water was shortened to a point that was insufficient to allow for normal larval development and metamorphosis for several species (Fig. 1). The duration of larval development in amphibians varies tremendously among amphibian species, from a few weeks in some anurans to several years in select stream salamanders (Duellman and Trueb 1986). Although the larval period within a species may vary substantially due to factors such as pond temperature and food levels (Scott 1990), among species variation in the duration of the larval stage also occurs due to intrinsic species differences in DNA content, metabolic rates, yolk reserves, and minimum size necessary for metamorphosis (Duellman and Trueb 1986). Our analysis showed that population trajectories were correlated with a species' response to hydroperiod; those that had increased frequency of recruitment failure due to insufficient hydroperiod were more likely to be declining. Declines were also likely influenced partly by competitive and predatory interactions (Semlitsch et al. 1996) as well as demographic factors, such as the unavailability of

mates even in years of suitable hydroperiod once populations crashed to very low levels (e.g., in 2003 and 2004 there were *A. tigrinum* females, but no males, at Rainbow Bay). As a result, the decline in these four species can be traced to the lack of reproduction that was induced by an increase in the number of years that were too dry for successful recruitment. It is possible that the drying trend at SRS during the 1990s is related to climate change. However, our analyses are insufficient to determine whether amphibian declines at SRS were ultimately driven by climate change, as has been previously proposed for other declining amphibian populations (Carey and Alexander 2003, Rohr and Madison 2003). Furthermore, we did not examine whether there is a linkage or synergy between the impact of a disease-causing agent and climate, as has been proposed for other declining amphibian populations (Pounds et al. 1999, Kiesecker et al. 2001).

Importantly, our findings demonstrate that the presence of *B. dendrobatidis* in a species assemblage does not always cause long-term declines of the component species. Host, pathogen, and environmental factors all influence the effects of *B. dendrobatidis* on amphibians (Daszak et al. 2003). Histological evidence suggests that the amphibian species present at SRS may be relatively resistant to infection by *B. dendrobatidis*. The small, focal lesions observed in *B. dendrobatidis*-infected SRS amphibians are markedly different to those reported from animals that died during chytridiomycosis-related mass mortality events (Berger et al. 1998). Small, focal lesions have also been reported in wild-caught introduced and captive-reared bullfrogs (*R. catesbeiana* [Mazzoni et al. 2003, Hanselmann et al. 2004]). In experimental studies, bullfrogs did not exhibit clinical signs of chytridiomycosis, despite inoculation with large numbers of *B. dendrobatidis* zoospores (Daszak et al. 2004). These data suggest that bullfrogs are relatively resistant to the disease chytridiomycosis, even though they can be infected by *B. dendrobatidis*. The findings in the current study could therefore be interpreted as evidence that wild *Rana* spp. frogs at SRS are also resistant to chytridiomycosis. Alternatively, the positive individuals could have been in the early stages of chytridiomycosis when captured. However, the lack of other positive specimens and lack of evidence of mass mortality suggest this is unlikely. Second, it is possible that the strain of *B. dendrobatidis* present at SRS is less virulent than those responsible for amphibian declines elsewhere. Isolation of the pathogen from SRS amphibians and future experimental infections may enable us to deduce which of these hypotheses explains the data. Finally, the drought conditions at SRS during this period may have themselves been unfavorable for this pathogen, i.e., ground temperatures may be too warm for too long a period annually to support efficient growth and spread of *B. dendrobatidis*, a pathogen that persists under relatively

cool conditions (Johnson et al. 2003, Piotrowski et al. 2004).

Our analyses also show that some species whose populations were not significantly declining have 10 or more consecutive years without successful reproduction (*B. terrestris*, *G. carolinensis*, and *S. holbrookii*). Species probability of persistence in the face of repeated catastrophic larval mortality due to early pond drying is related to species longevity (Taylor et al., *in press*). Since the average lifespan of *B. terrestris*, *G. carolinensis*, and *S. holbrookii* is thought to be less than 10 years (D. E. Scott, *unpublished data*), these data suggest that Rainbow Bay populations of these species are most likely part of larger metapopulations and are sometimes rescued by immigration (Semlitsch et al. 1996). This underscores the importance of considering the spatial scale for population analyses.

Our paper demonstrates the importance of analyzing long-term population and environmental data to determine causes of population declines (Pechmann et al. 1991) and the value of archived biological specimens to disease ecology studies. We showed that where amphibian populations decline and *B. dendrobatidis* is found, it is not always appropriate to assume that the pathogen is the cause of declines. This is especially important considering the now widespread use of PCR in surveying for *B. dendrobatidis* in amphibian populations, often without histopathological evidence of the pathogen's impact on individuals and without data on other infectious or non-infectious processes that can cause declines. Finally, it emphasizes an important feature of the ecology of infectious diseases—that the impact of even a seemingly virulent emerging pathogen can vary widely among species and populations and is affected by complex biotic and abiotic factors such as host resistance, strain virulence, and climate.

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APPENDIX

A figure showing histologic sections through the epidermis of a *Rana catesbeiana* from the Savannah River Site, South Carolina, USA, is available in ESA's Electronic Data Archive: *Ecological Archives* E086-175-A1.