An Aquatic Disease on a Terrestrial Salamander: Individual and Population Level Effects of the Amphibian Chytrid Fungus, *Batrachochytrium dendrobatidis*, on *Batrachoseps attenuatus* (Plethodontidae)

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The pathogenic chytrid fungus *Batrachochytrium dendrobatidis*, typically associated with anuran amphibians, is present in natural populations of the terrestrial salamander, *Batrachoseps attenuatus*, from California, USA, and four congeners from California and Oregon, USA. I demonstrate that the chytrid has been present in wild populations of *B. attenuatus* since at least 1973, and while infected salamanders collected in the wild exhibited 100% mortality in the laboratory, wild populations appear to have remained stable with seasonally variable infection rates. Laboratory experiments showed that inoculated salamanders housed in dry microhabitats, mimicking summer aestivation conditions, are able to shed the chytrid infection. Combining these data with the decrease in prevalence from spring to fall suggests that environmental conditions in the natural range of *B. attenuatus* mediate the effects of this potentially highly lethal pathogen, stabilizing this host–pathogen relationship. While *B. attenuatus* continues to be an abundant salamander, other amphibians are experiencing marked declines. An understanding of the relationship between the amphibian chytrid fungus and species of *Batrachoseps* may be applicable to patterns of declines and persistence in other species of plethodontid salamanders, and amphibians in general.

CHYTRIDIOMYCOSIS caused by the fungal pathogen *Batrachochytrium dendrobatidis* has been detected in amphibians worldwide and is a factor in global amphibian declines (Berger et al., 1998; Lips et al., 2006). *Batrachochytrium dendrobatidis* has a two-stage aquatic life cycle in which motile zoospores encyst on the keratinized epidermis of amphibians and develop into reproductive sporangia, which produce more zoospores (Berger et al., 2005). Although this chytrid is considered an aquatic fungus because it has water-dependent zoospores, it can survive up to 12 weeks in damp sterilized soil, and certain strains grow with moisture contents as low as 10% (Johnson and Speare, 2005). In culture, it is sensitive to both heat and desiccation, with mortality at temperatures above 32°C or when dried longer than three hours (Johnson et al., 2003).

Although some frog species appear to tolerate the chytrid (Daszak et al., 2004; Weldon et al., 2004), its presence has been correlated with high levels of mortality in many species (Stuart et al., 2004) and has been implicated in extinctions (La Marca et al., 2005). Environmental conditions can affect the outcome of the disease in frog populations; for example, infected *Litoria chloris* housed at high temperatures were capable of recovering from infection (Woodhams et al., 2003). Field surveys in Australian frog populations found that infection prevalence was lower in the warmer summer months, suggesting that environmental conditions, especially temperature, play an important role in determining fungal prevalence (Woodhams and Alford, 2005; Kriger and Hero, 2007a).

Although chytridiomycosis has been primarily studied in frogs, it also has been detected in several salamander families, including Ambystomatidae (Davidson et al., 2003), Amphiumidae (Speare and Berger, 2000), Cryptobranchidae (Briggler et al., 2008), Dicamptodontidae (USGS, 2001), Plethodontidae (Cummer et al., 2005), Proteidae (Speare and Berger, 2000), Salamandridae (Bosch and Martinez-Solano, 2006), and Sirenidae (Speare and Berger, 2000). Davidson et al. (2003) found the chytrid in *Ambystoma tigrinum* in southern Arizona, USA, but saw no mortality in experimentally infected individuals. In contrast, the findings of Bosch and Martinez-Solano (2006) suggested that chytridiomycosis could be implicated in the decline of *Salamandra salamandra* in Spain. These two species are aquatic breeders. The first reported case of the chytrid in a wild terrestrial plethodontid salamander was from a live *Plethodon neomexicanus*, collected in its natural habitat, in the Jemez Mountains of New Mexico (Cummer et al., 2005). Lips et al. (2006) found chytrid on dead salamanders from three direct developing plethodontid species in Panama: *Bolitoglossa schizodactyla*, *Oedipina collaris*, and *Oedipina* cf. *parvipes*, suggesting that the pathogen might have been a factor in the drastic decline of Central American plethodontid salamanders reported by Rovito et al. (2009), although there is limited evidence of infection in surviving populations.

Here, I describe the occurrence of the chytrid in populations of another terrestrial plethodontid salamander, *Batrachoseps attenuatus*, in northwestern California, USA, and in historical specimens of this species and four congeners, *B. gavilanensis*, *B. nigriventris*, *B. relictus*, and *B. wrightorum*, from California and Oregon, USA. These are direct-developing species that do not depend on water for any part of their life cycle. However, *Batrachoseps relictus* is unique among the sampled species, and unusual among congeners, as it can be found in water (Hansen and Wake, 2005). Species of *Batrachoseps* lay eggs in soil at the start of the rainy season and develop without a larval stage. Primarily nocturnal, these salamanders forage above ground on moist nights during the wet season from approximately mid-fall through spring, while spending the day in damp areas under cover objects or in leaf litter. In *B. wrightorum* the activity period begins later in the year, in April or May after the snow melts. During the dry summers, most species of *Batrachoseps* aestivate and do not reemerge until the first rains of the fall (Stebbins, 2003). Species of *Batrachoseps* are sedentary; individuals rarely travel distances greater than two meters (Hendrickson, 1952; Maiorana, 1978) and gene flow is extremely low even across relatively small geographic areas (Jockusch et al., 2001).

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The primary aim of this study was to investigate the effects of the chytrid at both the individual and population levels in *Batrachoseps attenuatus*. I tested contemporary field samples and historical museum specimens for presence of the pathogen and conducted two survival studies to evaluate the effects of the fungus on individual salamanders. The first survival study investigated mortality in *B. attenuatus* collected with the chytrid present, and the second investigated condition-specific mortality in inoculated *B. attenuatus* exposed to different moisture regimes. Additionally, a survey of historical specimens of selected congeners was conducted to assess the occurrence of the chytrid in similar taxa. Surveys of contemporary and historical species of *Batrachoseps*, combined with studies on the environmental conditions in which it can exist in these populations, provide information on how this chytrid affects populations of a terrestrial salamander species.

MATERIALS AND METHODS

Field studies.—The presence of the chytrid in *Batrachoseps attenuatus* was monitored through field surveys conducted intermittently from May 2006–May 2007 and monthly from October 2007–May 2008. Surveys conducted from 2006–2007 occurred at two sites in Berkeley, Alameda County (Strawberry Canyon Fire Trail ''SCFT'': 37.872497°N, 122.241696°W; and Haste Street, ''HS'': 37.865292°N, 122.262577°W). In the second year of the study, two sites, SCFT and SPD (South Park Drive, Berkeley, Alameda County, ''SPD'': 37.892748°N, 122.242363°W), were each visited monthly, on separate dates. Each survey yielded 15–30 *B. attenuatus* which were placed in clean, unused, plastic bags and released after sampling. Salamanders were sampled for chytridiomycosis by drawing a swab (Medical Wire and Equipment Co. MW113) repeatedly across the full ventral surface for 25–30 seconds. Surveys from October through March were conducted at night, under rainy conditions, when the salamanders were active on the surface. Because conditions were unusually dry during the later stages of the study period, all surveys after mid-April were diurnal and conducted by turning cover objects and searching leaf litter. Swabs were analyzed for the presence of *B. dendrobatidis* using quantitative (real-time) polymerase chain reaction (qPCR) techniques as described by Boyle et al. (2004).

Salamander collection.—*Batrachoseps attenuatus* for experiments were collected from two locations in the San Francisco Bay Area, California. Thirty were collected in a residential area in Berkeley, Alameda County (''HS'': 37.865292°N, 122.262577°W) from 25 May–17 June 2007, and swabbed within 2–10 days of collection for use in survivorship study I. An additional 40 were collected from an urban lot in San Francisco (37.73059°N, 122.42986°W) on 16 February 2008, and swabbed the day of collection for use in survivorship study II. All salamanders were housed in individual plastic containers in the Animal Care Facility at the University of California at Berkeley.

Survivorship study I.—Thirty *B. attenuatus*, collected from HS, were housed at 17–18°C, on damp paper towels, for up to 122 days. Nineteen of 30 (63%) of the salamanders tested positive for the chytrid and were designated as infected; the remaining 11 were labeled as controls. All salamanders were observed daily and swabbed weekly following the same swabbing protocol used in the field. Cages were cleaned weekly and each salamander was fed approximately five one-week-old crickets twice a week. Salamanders that died were preserved in 10% formalin. The study ended following the death of the last infected salamander.

Survivorship study II.—To determine the effects of moisture on progression of chytridiomycosis in *B. attenuatus*, 40 wild caught, chytrid negative salamanders were randomly assigned to one of four experimental groups. Twenty salamanders were exposed to isolate SW11, obtained from the autotomized tail of an infected *B. attenuatus* on 20 July 2007 and isolated using the protocols described in Longcore et al. (1999). Salamanders were inoculated using a modified version of the infection protocol described by Rachowicz and Briggs (2007). Inoculated salamanders were exposed to 3.0×10^9 zoospore-equivalents, quantified by qPCR, by placing each in a 15-mm plastic Petri dish lined with paper towels for three days, and pouring 10 ml of inoculum over the salamander into the dish. On the second and third day of the inoculation procedure the dishes were emptied and an additional 10 ml of fresh inoculum was added. Twenty control salamanders experienced an equivalent inoculation procedure using a sham inoculum collected from culture plates containing medium without the chytrid.

Ten randomly selected inoculated salamanders and ten randomly selected control salamanders were transferred to dry housing, and the remaining inoculated and control salamanders were transferred to wet housing. Wet housing consisted of containers at 100% relative humidity with a paper towel substrate saturated with approximately seven milliliters of water. Dry housing consisted of identical containers with modified lids allowing air flow between the room and the container to maintain 95% relative humidity. The dry-housed salamanders were kept at moisture conditions near the minimum level required for survival and were provided with a four-inch square of paper towel moistened daily with approximately one milliliter of water to prevent desiccation. All salamanders were checked daily, and weekly fed ten one-week-old crickets and cleaned. Salamanders were swabbed every other week and fatalities were either preserved in 10% formalin or frozen. Additionally, shed skin collected from infected salamanders ($n = 3$) was fixed to slides and stained following the same protocol described in the ''Historical sampling'' methods. The study was terminated after 110 days.

For survivorship studies I and II, mortality differences between groups were tested using the log rank test in JMP (version 2.0, SAS institute, Cary, NC, USA). Kaplan Meier curves were used to display survivorship functions. For survivorship studies I and II the growth rate (r) of zoospores (Z) on each individual salamander was calculated as the average rate of change in zoospore-equivalents ($r = (\ln(Z_{(t+1)}) - \ln(Z_t))/\Delta t$) and compared to zero using a one-tailed t-test.

Historical sampling.—Preserved specimens of *Batrachoseps attenuatus* (Contra Costa and San Mateo Counties, CA) from the Eastern and Southern clades (Jockusch et al., 2002), *B. gavilanensis* (Monterey County, CA), *B. relictus* (Tulare County, CA), *B. nigriventris* (San Louis Obispo County, CA), and *B. wrightorum* (Lane County, OR) were selected for histological examination for the chytrid based on written reports of salamander fatalities in the field or following collection, brown spots or markings on the skin,

Table 1. *Batrachochytrium dendrobatidis* Presence in *Batrachoseps attenuatus*. Field survey results, presented as number infected: number sampled, compiled by month (abbreviated as first letter) and site.

	Month	J	F	M	A	M	J	J	A	S	O	N	D
Year	Site												
2006	SPD	—	—	—	—	—	—	—	—	—	—	0:4	0:8
	SCFT	—	—	—	—	4:9	—	—	—	—	0:3	0:31	0:10
2007	SPD	—	—	—	—	—	—	—	—	—	0:20	0:26	0:17
	SCFT	1:7	—	—	—	0:3	—	—	—	—	0:17	0:27	0:39
	HS	—	—	—	—	29:44	—	—	—	—	—	—	—
2008	SPD	0:24	0:22	1:21	1:23	0:30	—	—	—	—	—	—	—
	SCFT	0:26	0:31	0:21	0:30	1:34	—	—	—	—	—	—	—
	HS	—	—	—	—	0:22	—	—	—	—	—	—	—

and autotomized tails (Maiorana, 1977; Field notes of Elizabeth L. Jockusch from the Archives of the Museum of Vertebrate Zoology, University of California, Berkeley). A 4-mm square of skin was removed from the ventral surface anterior to the cloaca, embedded in paraffin, sectioned at eight microns, and stained with hematoxylin and eosin (Humason, 1970). Slides were examined with a Nikon Optiphot-2 scope and photographs were taken using a Nikon Digital Sight DS-5M.

RESULTS

Field studies.—In initial surveys conducted in May 2006, four of nine *B. attenuatus* were chytrid-positive at SCFT. At HS in May 2007, 29 of 44 *B. attenuatus* were chytrid-positive. Between October 2007 and May 2008 three of the 345 *B. attenuatus* tested positive for the chytrid: one each in March, April, and May (Table 1).

Survivorship study I.—Nineteen of 30 (63%) salamanders collected at HS in May 2007 tested positive for the chytrid fungus using qPCR. Mortality was observed in 100% of the infected salamanders (Fig. 1, Kaplan Meier Curve, Log-rank test, $P < 0.001$). One salamander that was qPCR negative for the chytrid died at day 10 of unknown causes. One salamander in each of the infected and uninfected groups escaped on day 29 and were removed from the analysis at that date. Caudal autotomy, in which all or part of the tail was lost, occurred in four of the infected salamanders and was never seen in an uninfected individual; however, the difference was not significant (two-tailed Fisher's Exact Test, $P = 0.26$). Zoospore-equivalents, quantified using qPCR, increased with infection duration with the highest levels generally seen before death (Fig. 2). The growth rate of zoospores was calculated for each salamander and was significantly greater than zero (one-tailed Student's t-test, mean = 0.014, SE = 0.043, t = 3.15, $P = 0.0033$).

Survivorship study II.—Initially survivorship study II involved four groups of ten salamanders; however, laboratory accidents left five and seven salamanders in the inoculated and control dry groups, respectively. Despite the decrease in sample size, mortality patterns differed significantly between infected salamanders housed in wet and dry containers (Fig. 3, Kaplan Meier Curve, Log-rank test, $P = 0.04$). As in survival study I, infected salamanders that died generally exhibited a progressive increase in zoospore levels between inoculation and death (one-tailed Student's t-test, mean = 0.13, SE = 0.055, t = 2.36, $P = 0.032$). Zoospore-equivalents from salamanders in the dry infected group were highest

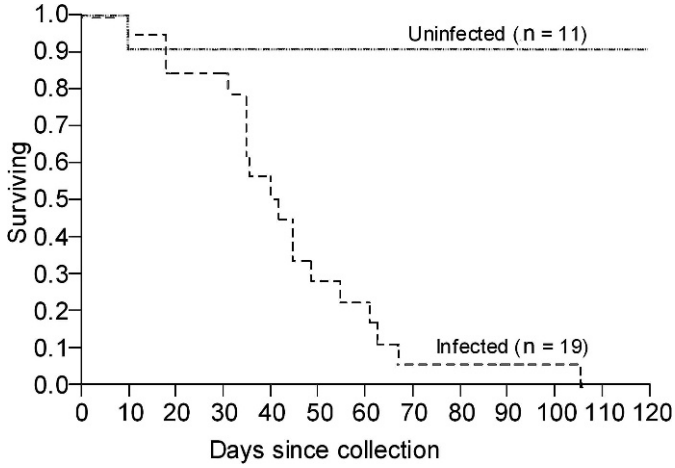

Fig. 1. Survival analysis of infected ($n = 19$) vs. uninfected ($n = 11$) *Batrachoseps attenuatus* collected in the field for survivorship study I. Kaplan Meier Curve, Log-rank test, $P = 0.0001$, censoring: 1 in each group at day 29. Day 0 represents the date of collection and the start of observations.

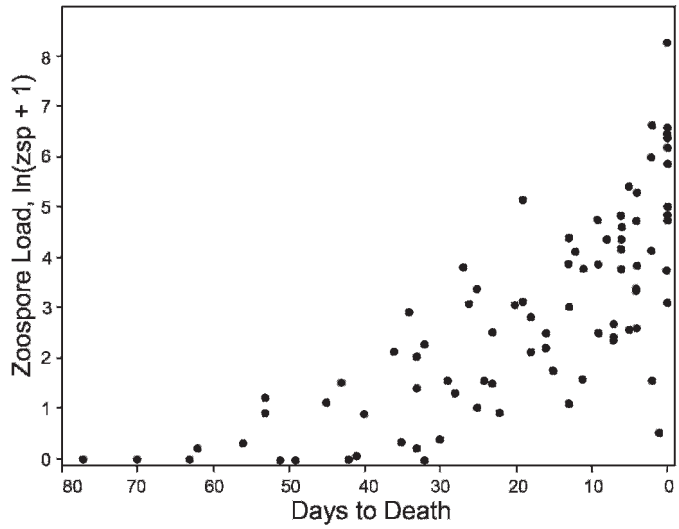

Fig. 2. Regression analysis of zoospore quantity by time to death in *Batrachoseps attenuatus* collected with naturally acquired *Batrachochytrium dendrobatidis* infection in survivorship study I. Zoospore growth rate is positive and significantly greater than zero (one-tailed Student's t-test, mean = 0.014, SE = 0.043, t = 3.15, $P = 0.0033$).

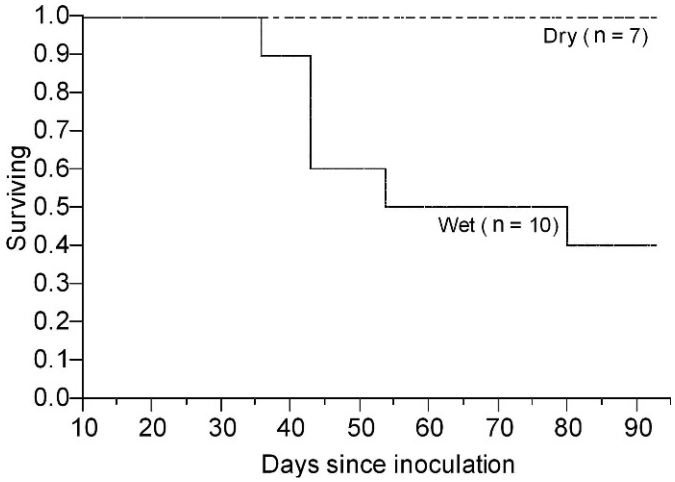

Fig. 3. Kaplan Meier Curve comparing survivorship between inoculated and control salamanders housed in wet and dry containers in survivorship study II. Uninfected controls are omitted from figure. (Log-rank test, $P = 0.04$).

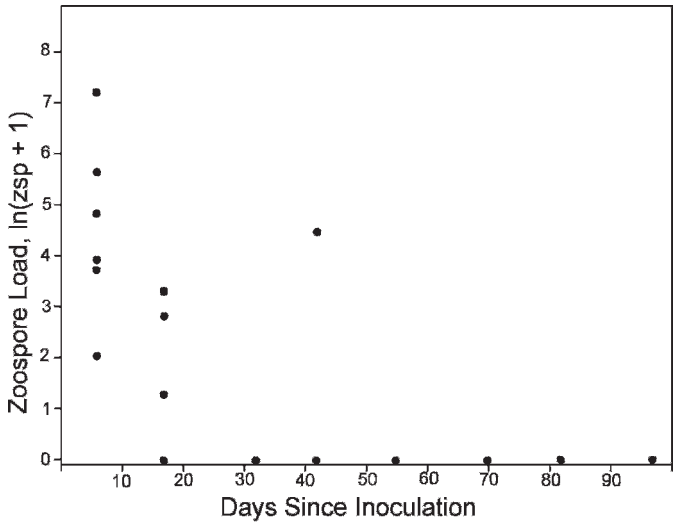

Fig. 4. Regression analysis of zoospore quantity by time since *B. dendrobatidis* inoculation in dry housed *B. attenuatus* in survivorship study II. Zoospore growth rate is negative and significantly less than zero (one-tailed Student's t-test, mean = −0.28, SE = 0.064, t = −4.41, $P = 0.007$).

immediately following inoculation and declined progressively over time showing a negative growth rate significantly less than zero (Fig. 4, one-tailed Student's t-test, mean = −0.28, SE = 0.064, t = −4.41, $P = 0.007$).

Morphological and histological observations.—Chytridiomycosis was detected in *Batrachoseps* specimens collected in 1973, 1974, 1993, 1994, and 1995 including *B. gavilanensis*, *B. relictus*, *B. nigriventris*, *B. wrightorum*, and *B. attenuatus* (Table 2).

In survivorship studies I and II dark brown spots were observed on the ventral surface of infected salamanders until shedding, at which time the dark patches were lost with the shed epidermis (Fig. 5). Histological examination of the preserved specimens showed that chytrid sporangia were localized within the darkened patches in those specimens in which spots were present ($n = 12$, Fig. 6). Whereas uninfected salamanders generally sloughed their skin in an entire piece, heavily infected animals generally sloughed fragmented skin in large quantities.

DISCUSSION

The amphibian chytrid fungus was present in populations of the terrestrial plethodontid salamander *Batrachoseps attenuatus*. Histological evidence shows that *B. dendrobatidis* has been infecting and likely killing these salamanders since at least 1973. Salamanders collected with a naturally acquired chytrid infection always died; however, wild populations of this species are not known to have experienced declines. Amphibian species exhibiting sharp declines (Rachowicz et al., 2006) have been shown to be susceptible to the chytrid fungus; however, in *B. attenuatus* high susceptibility in the laboratory is not associated with decreasing populations.

The earliest documentation of *Batrachochytrium dendrobatidis* in North America is in preserved specimens of *Rana clamitans* collected in Quebec, Canada in 1961. In California the earliest previous detection of the chytrid fungus was from specimens of *Rana muscosa* collected in 1975 (Ouellet et al., 2005). Results of my histological studies conducted on salamander specimens collected as early as 1973 showed clear evidence of chytridiomycosis. Its widespread presence and little evidence of natural mortality suggest a previously undetected, long-term association.

Rachowicz et al. (2006) documented drastic population declines in *Rana muscosa* following an initial outbreak of *Batrachochytrium dendrobatidis*. The lack of evidence of declines in populations of *B. attenuatus* may represent long-term persistence of the pathogen in these terrestrial salamanders. This suggests that this pathogen has been present in this region considerably longer than has been presumed. A more extensive examination of museum specimens might provide evidence of its annual spread or of a long-standing endemic relationship between salamander and fungus.

Extensive sampling efforts suggest that infected salamanders are uncommon but that sporadic outbreaks may occur. Between October 2007 and May 2008 only three of 345 salamanders sampled tested positive for *Batrachochytrium dendrobatidis*. In the 2007–2008 sampling period, rainfall decreased substantially after January leading to dry conditions in the last three survey months. If dry conditions limit infections in the laboratory, then similar conditions in the wild might drastically decrease chytrid prevalence. Less environmental moisture may limit the ability of the fungus to survive and infections to spread and infected salamanders may recover quickly due to environmental constraints on fungal growth.

In contrast to the generally low prevalence of *B. dendrobatidis*, 66% of the 44 *B. attenuatus* sampled at HS in May 2007 tested positive for the pathogen. Kriger and Hero (2007a, 2007b) documented a non-random distribution of the chytrid in breeding habitats of Australian frogs and found seasonal variations in infection prevalence and disease severity. In populations of *Batrachoseps* the chytrid appears to be sporadically present in the terrestrial environment, and high rates of infection seem spatially and temporally localized. Host density and dispersion affect transmission and prevalence (Rachowicz and Briggs, 2007; Rowley and Alford, 2007). In 2007 the HS site had a high salamander density, with upwards of ten salamanders sharing the same small cover objects. High densities should

Table 2. Historical Observations of Salamanders Exhibiting Symptoms Consistent with Chytridiomycosis and Results from Histological Examination. Specimens from the Museum of Vertebrate Zoology (MVZ).

Collection date	Species	Location	Observation	# Examined	# Positive
April 1971	*B. attenuatus*	Siesta Valley, Contra Costa Co., CA		9	0
1973	*B. attenuatus*	San Mateo Co., CA		12	12
April 1973	*B. attenuatus*	Moraga Canyon, Contra Costa Co., CA	40/40 collected died, caudal autotomy		
March 1974	*B. attenuatus*	Siesta Valley, Contra Costa Co., CA	2/47 collected died, brown spots on 7		
	B. attenuatus	Moraga Canyon, Contra Costa Co., CA	brown patches noted in field		
April 1974	*B. attenuatus*	Siesta Valley, Contra Costa Co., CA	5/96 dead in field, brown patches, caudal autotomy	3	3
	B. attenuatus	Moraga Canyon, Contra Costa Co., CA	2/14 dead in field		
Spring 1974	*B. attenuatus*	Berkeley. Alameda Co., CA	"hundreds" collected, "most of which died"		
February 1993	*B. gavilanensis*	Monterey Co., CA	4, caudal autotomy; 1, dead	3	3
March 1993	*B. nigriventris*	San Louis Obispo Co., CA	7, caudal autotomy	3	3
May 1993	*B. wrightorum*	Lane Co., OR	1, caudal autotomy		
April 1994	*B. gregarius*	Mariposa Co., CA	1, caudal autotomy		
June 1994	*B. wrightorum*	Lane Co., OR	1, caudal autotomy	1	1
April 1995	*B. gregarius*	Mariposa Co., CA	2, caudal autotomy		
August 1995	*B. relictus*	Tulare Co., CA	9, died after collection	3	1

increase the likelihood of contacts between salamanders. Additionally, if zoospores can survive briefly in the environment (Johnson and Speare, 2003, 2005), then high host density would increase the likelihood of salamanders contacting shed zoospores or shed skin containing infectious zoospores. The relationship between substrate moisture and chytrid infection may be further complicated by salamander densities and habitat quality, as species of *Batrachoseps* prefer damp substrate and aggregate in suitable habitat (Hendrickson, 1952).

Preliminary field data and older reports of mortality in species of *Batrachoseps* suggest a seasonal pattern of chytrid prevalences. Jockusch (Field notes from the Archives of the Museum of Vertebrate Zoology, University of California, Berkeley) and Maiorana (1974, 1977) observed salamander mortality in the field and laboratory coupled with symptoms consistent with chytridiomycosis. Histological analyses done on a subset of the salamanders collected at the time of these observations showed evidence of the fungus. In species of *Batrachoseps*, episodes of mortality were primarily restricted to the months of February through May and appear to conform to seasonal trends (Berger et al., 2004; Kriger and Hero, 2007a). Amphibian declines and associated mortalities have often been documented at cooler, high elevation sites, suggesting a relation between temperature and disease prevalence (Berger et al., 1998; Bradley et al., 2002).

In populations of *Batrachoseps* the absence of infections in the fall but their presence in the spring suggest that, over the summer, infected salamanders are either succumbing to or are eliminating infections. Elevated body temperature can cure infections (Woodhams et al., 2003). However, summer temperatures within the study region rarely exceed the thermal limits of *B. dendrobatidis*. Although the temperature is well within the range required for chytrid growth, moisture levels may not be. All infected salamanders held in dry laboratory conditions decreased zoospore load and ultimately lost their infections, suggesting that infected *B.*

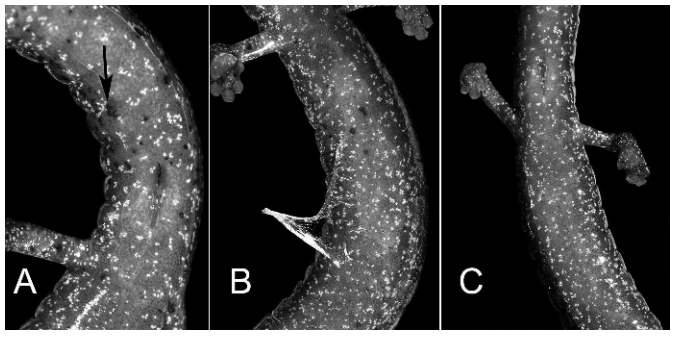

Fig. 5. A series of photographs of a single infected *Batrachoseps attenuatus* over a ten-minute observation period. Dark brown spots (example marked with arrow) are initially visible on the ventral surface of the salamander (A). The outer layer of skin is shed in fragments (B). Following shedding no dark marks are visible on the salamander (C). Photographs by Anand Varma.

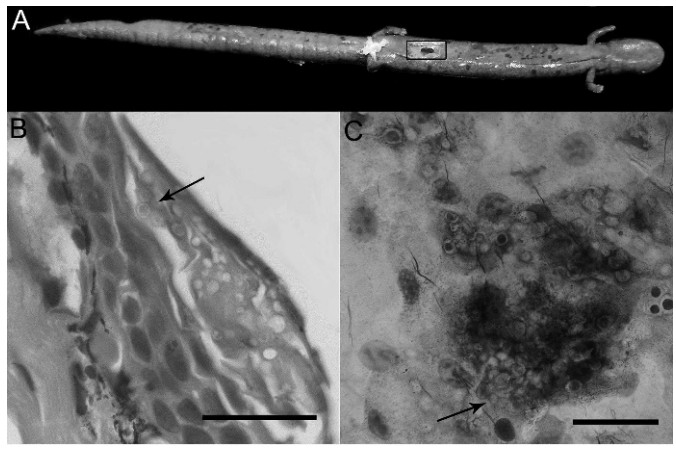

Fig. 6. *Batrachochytrium dendrobatidis* infecting *Batrachoseps attenuatus*. (A) Specimen collected in 1974 in Contra Costa County, with brown spots characteristic of *Bd* infection. Box demarcates area of skin removed for histological examination. (B) Histological section of skin showing zoosporangia (arrow) under melanized spot. Scale bar = 50 μm. (C) Sloughed skin showing concentration of chytrid thalli (arrow), at various stages of development, within melanized spot. Scale bar = 50 μm.

attenuatus may recover during summer aestivation. Dry housing also may be effective to treat infected captive amphibians.

While dry years might lead to low disease prevalence, unusually wet years may cause an upsurge in parasitization. The years when salamanders were found dying in the field (1973–1974) were unusually wet, with rainfall more than one standard deviation above average (National Climatic Data Center, http://www.ncdc.noaa.gov/oa/ncdc.html). Unusually wet conditions may account for how salamanders could reach such high infection levels and density in the field. Prolonged wet periods could cause an increase in abundance of *Batrachoseps attenuatus* by increasing the foraging time for hatchlings prior to aestivation, thus increasing survival (Maiorana, 1976). Combining favorable fungus conditions with a population boom may explain the large number of salamanders found dead and dying in the field, as reported in 1973 and 1974 (Maiorana, 1977). The reports of mortalities in species of *Batrachoseps* and environmental constraints on fungal growth suggest that multiyear, large scale surveys of this genus (and sympatric amphibians) may reveal a more complicated pattern of chytrid prevalence.

Death in these lungless salamanders may be due to profound disruption of normal physiological processes from the destruction and loss of the epidermis. Voyles et al. (2007) found that *Litoria caerulea* with severe clinical signs of chytridiomycosis had reduced electrolyte levels and suggested that chytrid disrupts cutaneous transport. As observed for *B. attenuatus* in this study, excessive or abnormal shedding has been documented in chytrid infected frogs (Berger et al., 1998, 2005; Pessier et al., 1999) and salamanders (Davidson et al., 2003; Padgett-Flohr, 2008), suggesting that animals may be actively responding to infection by increased shedding. Frequent shedding may be a normal physiological mechanism for decreasing fungal load and under unfavorable conditions for fungal growth may allow an infected salamander to dispel the pathogen faster than the chytrid can grow. However, under wet conditions favoring fungal growth, the pathogen might grow faster than it can be shed, leading to substantial tissue damage if the salamander continues to slough its skin at a high rate. These preliminary observations suggest that skin sloughing should be investigated as a defensive mechanism to survive fungal infections in terrestrial salamanders.

Caudal autotomy is generally considered an adaptation to avoid predation (Wake and Dresner, 1967); however, this behavior was seen in salamanders with severe chytridiomycosis, suggesting that disease can also cause tail loss (Table 2, survivorship study I). Dark spots were observed on the ventral surface of infected *Batrachoseps*, both in the field and in museum specimens (Figs. 5, 6). Similar spots have been observed in other infected salamanders: *Ambystoma tigrinum*, *Euproctus platycephalus*, and *Plethodon neomexicanus* (Davidson et al., 2003; Cummer et al., 2005; Bovero et al., 2008). In species of *Batrachoseps*, these spots, and caudal autotomy, were good indicators of chytrid presence, especially in preserved specimens. This detection method might be useful in other salamander species.

The amphibian chytrid fungus is present in populations of *Batrachoseps* and can induce high levels of mortality in this abundant terrestrial salamander. Chytrid infections in populations of these sedentary salamanders suggest that the pathogen may be present, and possibly widespread in soil. Because a decrease in moisture leads to a loss of infection in this species, terrestrial amphibians in seasonally dry environments may be more likely to persist despite fungal presence. Additionally, the physiological response of skin shedding may be an important survival mechanism. While populations of *B. attenuatus* do not appear to be strongly affected by the presence of *B. dendrobatidis*, the study of this pathogenic fungus in this species reveals physiological and environmental factors that may affect the survival and, ultimately, persistence of declining amphibian species.

MATERIAL EXAMINED

Batrachoseps attenuatus: MVZ 258459–258484, 258417–258458.
Batrachoseps gavilanensis: MVZ 224681, 224693, 224695.
Batrachoseps nigriventris: MVZ 22462, 224634, 224636.
Batrachoseps relictus: MVZ 224821, 224825, 224826.
Batrachoseps wrightorum: MVZ 224877.

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