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Amphibian Chytrid (*Batrachochytrium dendrobatidis*) in Post-Metamorphic *Rana boylii* in Inner Coast Ranges of Central California

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In California, USA, the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), has been reported to infect many species of native ranids (Nieto et al. 2007; Padgett-Flohr 2007) with particular attention being focused on dramatic *Bd*-related declines of the Sierra Nevada Yellow-legged Frog (*Rana sierrae*) in the Sierra Nevada mountains (Davidson et al. 2007; Fellers et al. 2001; Rachowicz et al. 2006). The Foothill Yellow-legged Frog (*R. boylii*) is a close relative of *R. sierrae* that occurs in streams over much of California, but at lower elevations (<1940 m) than *R. sierrae* (Fellers 2005). In 2006, *Bd* was found in *R. boylii*, Pacific Treefrogs (*Pseudacris sierra* sensu Recuero et al. 2006a, b), and Western Toads (*Anaxyrus boreas*) at Pinnacles National Monument, managed by the US Department of Interior - National Park Service, and in nearby streams on lands managed by the US Department of Interior - Bureau of Land Management (BLM) in San Benito and Fresno counties (P. Johnson, pers. comm.). The BLM in California recognizes *R. boylii* as a sensitive species, and it is a California Species of Concern (http://www.dfg.ca.gov/wildlife/species/ssc/amphibians.html). I surveyed *R. boylii* from nine streams in this region and tested them for *Bd* to better understand the geographic distribution of the pathogen. Incidental captures of *P. sierra* also were tested. In addition, I conducted a preliminary investigation of potential differences in habitat associations of infected and non-infected *R. boylii*.

Methods.—Between 5 July and 14 September 2006, post-metamorphic *R. boylii* and *P. sierra* were opportunistically caught during abundance counts conducted using visual encounter surveys (Fellers and Freel 1995) along streams in the Inner Coast Ranges of central California (Fig. 1). During abundance counts, frogs were recorded as adults or subadults subjectively based on size. Skin swabs were collected from each captured frog and screened for *Bd* presence using PCR amplification (Pisces Molecular LLC, Boulder, Colorado, USA). Sterilized equipment and disposable gloves were used for each frog handled. Snout–vent length (SVL), weight, and sex were recorded for each frog. For each capture location, microhabitat variables were recorded including water temperature, water depth, stream width, water velocity, stream habitat unit type (riffle, run, pool, step-pool), dominant substrate size (modified from Platts et al. 1983; sand [<2 mm diam], gravel [2–63 mm], cobble [64–256 mm], boulder [>256 mm]), dominant riparian vegetation, and location by Universal Transverse Mercator. Two-sample t-tests were used to compare continuous variables (SVL, weight, SVL-to-weight ratio, water temperature, water depth, and stream width) between *Bd*-negative and *Bd*-positive samples using NCSS 2000 statistical software. To assess frog condition between those animals with and without *Bd*, SVL-to-weight ratios were compared. Categorical variables (sex, stream habitat unit, substrate, and vegetation) did not yield adequate within-group samples sizes for statistical analysis, hence summaries for these variables are given.

Results.—Forty-nine *R. boylii* were tested for *Bd* from nine streams, and seven *P. sierra* were tested from three streams and a BLM vehicle wash facility. Nine of 49 (18%) *R. boylii* and one of seven (14%) *P. sierra* tested positive for *Bd*. Five of nine (56%) streams supporting *R. boylii* had at least one frog testing positive for *Bd* (Fig. 1, Table 1). Different streams were tested during different months of the year. All streams tested in July had *Bd*-positive frogs, as did half the streams tested in August, but no streams tested in September had *Bd*-positive frogs. Frogs at one stream, White Creek, tested positive for *Bd* during pilot sampling in May but tested negative in September. *Bd*-positive frogs occurred in streams with low *R. boylii* densities (e.g., 41 post-metamorphic frogs/km) to high *R. boylii* densities (e.g., 527 post-metamorphic frogs/km, Table 1). The single *Bd*-positive *P. sierra* was captured at Sawmill Creek where one *R. boylii* was also *Bd*-positive. The remaining *P. sierra*, including two captured from the vehicle wash facility, tested negative for *Bd*.

Rana boylii of all size classes (15–75 mm SVL) were tested, but only smaller frogs (<41 mm SVL) tested positive for *Bd* (Fig. 2). Although there was no significant difference in SVL between *Bd*-positive and *Bd*-negative groups ($p = 0.1260$, $t = 1.558$), *Bd*-positive frogs weighed less (mean *Bd*+ = 3.4 ± 0.6 SE, N = 8;

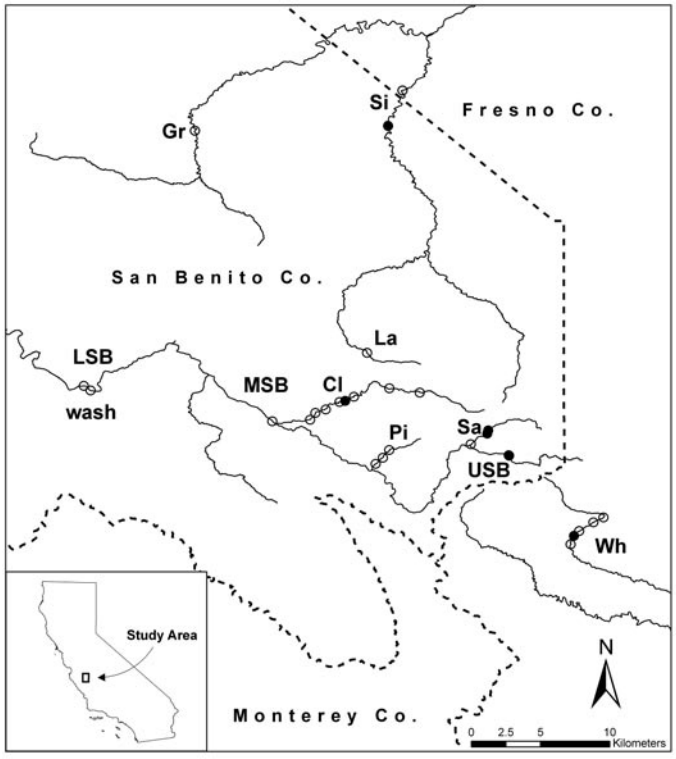

FIG. 1. Locations of positive (black circles) and negative (open circles) test results for *Batrachochytrium dendrobatidis* in *Rana boylii* and *Pseudacris sierra* in Griswold Creek (Gr), Silver Creek (Si), Larious Creek (La), lower San Benito River (LSB), vehicle wash facility (wash), middle San Benito River (MSB), Clear Creek (Cl), Picacho Creek (Pi), Sawmill Creek (Sa), upper San Benito River (USB), and White Creek (Wh), California, USA.

TABLE 1. Streams surveyed for *Rana boylii* density (frogs/km) and results of *Batrachochytrium dendrobatidis* (*Bd*) tests in 2006 in southern San Benito and western Fresno counties, California, USA.

Stream Name	Adult Density	Sub-adult Density	No. Frogs Tested for *Bd*	No. *Bd*-Positive Frogs	Date of Samples
White Creek	92	435	11	0[a]	8 Sept
Clear Creek	54	205	14	1	27 July
San Benito River (upper)	53	54	6	4	12 July
Picacho Creek	25	153	5	0	1,5 Sept
Larious Creek	14	0	2	0	9 Aug
Sawmill Creek	12	9	2	1[b]	5,7 July
Silver Creek	8	33	6	3	17 Aug
San Benito River (lower)	2	3	2	0	7 Sept
San Benito River (middle)	0	9	1	0	7 Sept

[a] Although negative for *Bd* in September, a pooled sample of 10 *Rana boylii* from White Creek tested positive for *Bd* during pilot sampling on 9 May 2006.
[b] One *Pseudacris sierra* from Sawmill Creek was *Bd*-positive.

mean *Bd*- = 8.8 ± 1.2 SE, N = 39; p = 0.0282, t = 1.958). Smaller frogs (< 41 mm SVL) testing positive for *Bd* had higher SVL-to-weight ratios than frogs of the same size class testing negative for *Bd* (mean *Bd*+ = 8.0 ± 0.5 SE, N = 5; mean *Bd*- = 6.9 ± 1.0 SE, N = 16; p = 0.0174, t = -2.274). Frogs > 41 mm SVL were not included in this analysis. Because of their small sizes, none of the nine *R. boylii* testing positive for *Bd* could be sexed. Frogs that tested positive were not symptomatic for chytridiomycosis, and no frogs seen during abundance counts appeared abnormal in any way. However, one sub-adult *R. boylii* was found dead at the bottom of White Creek being consumed by a stink bug (Pentatomidae).

Bd-positive frogs were found in streams with various types of riparian vegetation (e.g., Brewer's Willow [*Salix breweri*], Salt Cedar [*Tamarix ramosissima*], and California Sagebrush [*Artemisia californica*]) and in various aquatic habitat conditions, but primarily in pools with fine substrates and cooler than average water. Eight of the nine (89%) *Bd*-positive *R. boylii* and the *Bd*-positive *P. sierra* were caught in pools, even though pools made up only 43% of the frog capture locations. The other *Bd*-positive *R. boylii* was captured in a high-velocity riffle with a gravely substrate from Clear Creek. All the *Bd*-positive pools had sandy substrates except those on the upper San Benito River, which had cobble-dominated substrate. Water temperatures were lower at *Bd*-positive sites (mean *Bd*+ = 20.0 ± 1.6 SE, N = 9; mean *Bd*- = 23.7 ± 0.5 SE, N = 40; p = 0.0102, t = 2.678). When riffles and runs are taken out of the analysis to compare water temperatures of pools only, the pattern of cooler temperatures at sites with *Bd*-positive animals remains significant (mean *Bd*+ = 20.2 ± 1.8 SE, N = 8; mean *Bd*- = 23.3 ± 0.8 SE, N = 13; p = 0.0475, t = 1.757). Stream width (range 0.5–4.5 m) and water depth (4–68 cm) did not differ between sites with *Bd*-positive and *Bd*-negative frogs (p = 0.7421, t = 0.3310; p = 0.4668, t = 0.7337, respectively).

Discussion.—I detected *Bd* in 18% of the *R. boylii* sampled, and in 5 of 9 streams in southern San Benito and western Fresno counties, California. The small sample size from some streams might explain lack of detection, given the relatively low prevalence rate across all sampled frogs. Interestingly, none of the streams sampled in September resulted in *Bd*-positive results even though one of these was known to have *Bd*-positive frogs in May. Other studies have shown that higher prevalence and mortality rates of *Bd* are detected in winter and early spring than in late summer and early fall (Berger et al. 2004; Kriger and Hero 2006, 2007). Streams in the present study were sampled during various months of the summer, and a seasonal effect may have confounded analyses. If a seasonal effect resulted in false negatives for frogs tested in September, then the pattern of *Bd* distribution in the study area and the overall prevalence rate described here could be underestimated.

I found that only *R. boylii* less than 41 mm SVL and weighing less than 4.0 g tested positive for *Bd*. Frogs of this size are young-of-the-year or sub-adults in their second post-metamorphic year (Stebbins 1962; Storer 1925; Zweifel 1955). These younger age classes had reduced body condition (as estimated by SVL-to-weight ratio) if they tested positive for *Bd*. No adults in this population tested positive for *Bd*, which might indicate a difference in *Bd* susceptibility between these life history stages. Desiccation and temperatures over 30ºC are known to be lethal to Bd (Daszak et al. 2000; Drew 2006; Johnson et al. 2003), and *R. boylii*, especially adults (pers. obs.), are known to bask on open

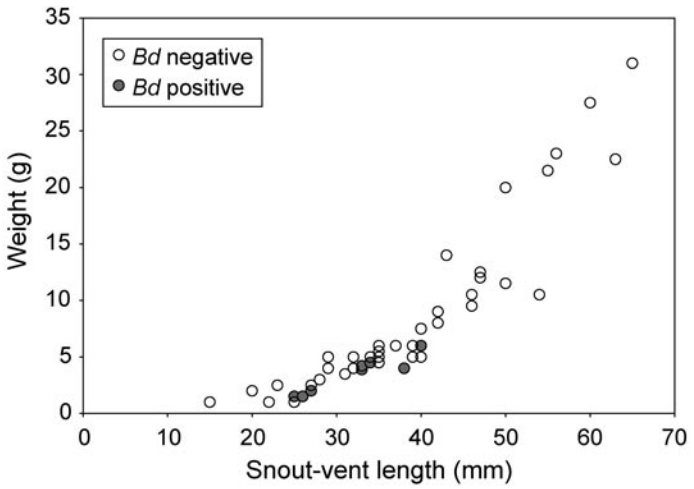

FIG. 2. Sizes of 48 *Rana boylii* tested for *Batrachochytrium dendrobatidis* (*Bd*) in California, USA. *Bd*-positive individuals (N = 8) are shown in black. One *Bd*-positive frog (33 mm SVL) was not weighed.

sunny stream banks with less than 20% canopy cover (Fuller and Lind 1992; Stebbins 1962). This behavior might prevent *Bd* from infecting *R. boylii* adults or help them to shed the fungus if infected, especially in the foothills of California where summer air temperatures commonly exceed 35ºC. Although, mortality of *Bd*-infected adults cannot be ruled out, no evidence for adult chytridiomycosis or mortality was seen in this study.

Pool habitats and cooler water temperatures were the only habitat variables that were significantly associated with the presence of *Bd*. Pools provide rearing habitat for *R. boylii* larvae, and in one study, *R. sierrae* larvae have been shown to transmit *Bd* to post-metamorphic frogs (Rachowicz and Vredenberg 2004). The preponderance of *Bd*-positive frogs sampled from pools where larvae tend to congregate appears to support Rachowicz's suggestion that larvae and thus their habitats might serve as reservoirs for the fungus. It is uncertain whether the finding that *Bd*-positive frogs were sampled from areas with cooler water temperatures than *Bd*-negative frogs has any biological significance because the difference in water temperatures between these groups was small (20ºC versus 24ºC respectively) and within *Bd*'s optimal growth range of 17–25ºC (Daszak et al. 2000; Piotrowski et al. 2004).

Populations of *R. boylii* appear to be stable in streams involved in this study (BLM, unpubl. data, 2005–2007). The fact that large populations of *R. boylii* are present in streams with *Bd*-infected animals without showing declines or symptoms of chytridiomycosis is encouraging. However, if *Bd* is causing reduced body condition in younger age classes, population sizes might eventually be affected. This study was conducted over only one year, with small sample sizes, and might have been confounded by seasonal effects. I suggest future sampling be temporally stratified across seasons and years, with greater sample sizes of frogs tested. Furthermore, population monitoring to determine the lethality of *Bd* on various life history stages, prevalence rates, and population-level effects in various streams over time is warranted given the sensitive status of the species and the effects the disease has had on its close relative *R. sierrae*.

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