Effects of the Amphibian Chytrid Fungus and Four Insecticides on Pacific Treefrogs (Pseudacris regilla)

Article *in* Journal of Herpetology · December 2012

DOI: 10.2307/23327184

CITATIONS
13

READS
141

3 authors, including:

Peter Kleinhenz
Tall Timbers
6 PUBLICATIONS **18** CITATIONS

SEE PROFILE

Michelle Boone
Miami University
85 PUBLICATIONS **2,750** CITATIONS

SEE PROFILE

Journal of Herpetology, Vol. 46, No. 4, 625–631, 2012

Effects of the Amphibian Chytrid Fungus and Four Insecticides on Pacific Treefrogs (*Pseudacris regilla*)

Peter Kleinhenz,[1] Michelle D. Boone,[1,2] and Gary Fellers[3]

[1]212 Pearson Hall, Department of Zoology, Miami University, Oxford, Ohio 45056 USA
[3]U.S. Geological Survey, Western Ecological Research Center, Point Reyes National Seashore, Point Reyes, California 94956-9799 USA

Abstract.—Chemical contamination may influence host-pathogen interactions, which has implications for amphibian population declines. We examined the effects of four insecticides alone or as a mixture on development and metamorphosis of Pacific Treefrogs (*Pseudacris regilla*) in the presence or absence of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis* [Bd]). Bd exposure had a negative impact on tadpole activity, survival to metamorphosis, time to metamorphosis, and time of tail absorption (with a marginally negative effect on mass at metamorphosis); however, no individuals tested positive for Bd at metamorphosis. The presence of sublethal concentrations of insecticides alone or in a mixture did not impact Pacific Treefrog activity as tadpoles, survival to metamorphosis, or time and size to metamorphosis. Insecticide exposure did not influence the effect of Bd exposure. Our study did not support our prediction that effects of Bd would be greater in the presence of expected environmental concentrations of insecticide(s), but it did show that Bd had negative effects on responses at metamorphosis that could reduce the quality of juveniles recruited into the population.

Low-levels of environmental contamination are prevalent even in areas once considered pristine (McConnell et al., 1998; Sparling et al., 2001; Angermann et al., 2002). Contaminants, therefore, are additional factors in most systems already exposed to competition, predation, pathogens, and important abiotic factors that regulate populations and communities (Boone and Bridges, 2003). Because chemical contaminants can change an individual's response to other stressors (reviewed in Relyea and Hoverman, 2006), they have the potential to influence population dynamics as well as species persistence. Global amphibian population declines are attributed to many factors that threaten biodiversity, including habitat destruction, contamination, invasive species, overexploitation, disease, or combinations of multiple factors (Houlahan et al., 2000; Stuart et al., 2004). Disease, however, appears to be a major cause of enigmatic declines that have no other apparent cause (reviewed in Fisher et al., 2009; Collins and Crump, 2010). Because of the pervasiveness of contamination in environments and because of their potential to immunosuppress organisms (Blakley et al., 1999), understanding the significance of contamination in altering host-pathogen dynamics is of great importance because it offers potential management solutions such as reduced pesticide use or greater pesticide regulation.

The amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (Bd), is a widespread pathogen (Daszak et al., 2005; Longcore et al., 2007; Skerratt et al., 2007; Rothermel et al., 2008) and has been associated with amphibian population declines and extinctions around the world (Berger et al., 1998; Daszak et al., 2003; Muths et al., 2003; Lips et al., 2008; Vredenburg et al., 2010). Infection by Bd can be asymptomatic or result in the disease of chytridiomycosis, characterized by excessive skin shedding and osmotic imbalance that can result in death (reviewed in Fisher et al., 2009). Amphibians show variation to Bd susceptibility within and among populations (Briggs et al., 2005; Tobler and Schmidt, 2010) and between species (Blaustein et al., 2005, reviewed in Fisher et al., 2009; Stockwell et al., 2010). The observed variation in response to Bd may result from genetic or immunological differences, as well as the presence of other environmental stressors that may increase or decrease vulnerability.

[2]Corresponding Author. E-mail: boonemd@muohio.edu
DOI: 10.1670/11-070

Presence of pesticides, which have been correlated with amphibian population declines in some areas (Davidson et al., 2001, 2002; Sparling et al., 2001; Davidson, 2004), have the potential to interact with pathogens as well as other factors to compromise amphibian immunity (Carey and Bryant, 1995; Hayes et al., 2010). Environmental field concentrations of most pesticides are sublethal to amphibians, but sublethal concentrations can turn lethal or compromise amphibians in the presence of predators (Relyea and Mills, 2001; Relyea, 2003), competitors (Boone and Semlitsch, 2001; Mills and Semlitsch, 2004; Distel and Boone, 2010), or pathogens (Parris and Baud, 2004; Davidson et al., 2007; Hayes et al., 2006; Rohr and Raffel, 2010). Pesticide exposure can also increase the likelihood of disease or infection (Taylor et al., 1999; Christin et al., 2003; Gendron et al., 2003; Hayes et al., 2006; Davidson et al., 2007). For instance, Davidson et al. (2007) found that juvenile foothill Yellow-legged Frogs (*Rana boylii*) produced skin peptides that inhibited Bd growth in vitro; however, the amount of skin peptides produced was reduced in the presence of the insecticide carbaryl, which could make frogs more vulnerable to other environmental stressors like pathogens. Because skin peptides, as well as cutaneous bacteria, appear to inhibit pathogens including Bd (Harris et al., 2006; Woodhams et al., 2007), factors that reduce the efficacy of these natural defenses put individuals at risk. Understanding the potential for the threats of pesticides and pathogens to singly or interactively influence amphibian populations is of critical importance to determine what actions could slow or prevent further declines.

Our objective was to examine the potential interactions between Bd and insecticides on larval development through metamorphosis using Pacific Treefrogs (*Pseudacris regilla*). To do this, we exposed Pacific Treefrog tadpoles to expected environmental concentrations of malathion, diazinon, endosulfan, chlorpyrifos, or a mixture of all four insecticides in the presence or absence of Bd to determine impacts on metamorphosis. We selected Pacific Treefrogs because they were susceptible to Bd in some studies (Romansic et al., 2011; although not in others, Garcia et al., 2006), because they can act as a reservoir for the pathogen while remaining asymptomatic (Conlon, 2011), and because we wanted to use a surrogate species from areas of declines that was not at risk. We selected the insecticides malathion, diazinon, chlorpyrifos, and endosulfan because they are used commonly and found in areas inhabited by Pacific

Treefrogs (Kuivila and Foe, 1995; McConnell et al., 1998; Sparling et al., 2001). Additionally, Fellers et al. (2007) found amphibians breeding at sites in California where both chemical contaminants and Bd were present. Because the concentrations we used were below levels expected to affect survival or metamorphosis (Sparling and Fellers 2007, 2009; Widder and Bidwell, 2008; Relyea 2009), we predicted that sublethal concentrations of insecticides alone would have no effect or small negative effects. Additionally, we predicted that Bd would have negative impacts on metamorphosis and that the combination of Bd and insecticide(s) would interact synergistically to have negative impacts on tadpole activity, survival to metamorphosis, mass to metamorphosis, time to metamorphosis, and feeding ability of Pacific Treefrogs.

Materials and Methods

Three egg masses of Pacific Treefrogs each containing ~70 eggs were collected from a seasonal pond (site P-204) at Point Reyes National Seashore in Marin County, California on 21 February 2010 and shipped overnight on wet ice to Miami University in Oxford, Ohio. Egg masses were held in the laboratory at 20°C on a 12 : 12 light : dark cycle in water from the collection site until hatching. Mixed clutches of tadpoles were transferred to charcoal-filtered water and fed ground TetraMin® Tropical Fish Flakes ad libitum; water was changed daily.

To create the Bd solution, we ordered two plates of Bd isolate JEL626 on nutrient agar on 15 January 2010 from J. Longcore (University of Maine). The isolate originated from a young Bullfrog (*Lithobates catesbeianus*) caught in California in October 2009, which is within the range of Pacific Treefrogs. We made a 1% tryptone broth (poured from a solution of 10 g tryptone and 1,000 mL distilled water) that was sterilized and cooled before placing a 2-mm^2 piece of the agar from the cultured isolate in 75 mL of the broth. After clumps of thalli were visible in the broth, we added 1 mL of the broth culture to each of 10 plates of sterilized tryptone agar (from a solution of 10 g tryptone, 10 g agar, and 1,000 mL distilled water) on 3 and 23 February. We kept plates in the lab at 22–24°C and held until zoospores were visible at 10 × magnification. A Bd solution was made by rinsing the inoculated plates with aged tap water.

We manipulated exposure of tadpoles to presence or absence of Bd and one of six insecticide exposures (water control, 75 μg/L diazinon, 30 μg/L malathion, 4 μg/L chlorpyrifos, 0.2 μg/L endosulfan, or a mixture of all of these insecticides at the given concentrations) with 17 replicates of each treatment (2 Bd treatments × 6 pesticide treatments × 17 replicates = 204 individually reared tadpoles); additionally, we had nine acetone controls, which served as negative controls to assess whether any pesticide effects were attributable to the carrier (acetone) of the insecticides. On 15 March 2010 (experimental day 0), tadpoles were placed individually in 100 mL of dechlorinated water in a 1-L glass beaker and were exposed to 1 mL of aged tap water or 1 mL of Bd solution (~2.78×10^6 zoospores/mL) for 24 h, which resulted in an exposure of 27,800 zoospores per 100 mL; tadpoles were exposed in a low volume of water to increase the rate of exposure to Bd (similar to Venesky et al., 2010a,b).

After 24-h exposure to the Bd treatment, we added an additional 900 mL of water to all beakers to reach 1 L and applied insecticide treatments. Insecticide exposures represented approximately 1% of reported LC50s for anurans based on previous studies (cited below), allowing each insecticide to represent an equivalent toxic unit. We used 1% of the LC50 as our criteria because Bridges (2000) found >70% mortality in Southern Leopard Frogs (*Lithobates sphenocephalus*) reared with carbaryl (insecticide) exposure of 4–10% 96 h LC50; however, with exposure at 1.5% of the LC50, survival was not significantly different than control, and exposure influenced time and size to metamorphosis. Additionally, the 1% LC50 was similar to concentrations found in the natural habitat of Pacific Treefrogs (Sparling and Fellers, 2007, 2009), which were not expected to have effects on performance based on previous studies (Sparling and Fellers, 2007, 2009; Relyea, 2009). We added 0.25 mL of prepared stock solutions to 1 L of water for each insecticide to reach 75 μg/L diazinon (362 mg diazinon [0.995 purity] added to 1,200 mL acetone; based on Sparling and Fellers, 2007), 30 μg/L malathion (146 mg malathion [0.986 purity] added to 1,200 mL acetone; based on Relyea, 2004), 4 μg/L chlorpyrifos (19.3 mg chlorpyrifos [0.995 purity] added to 1,200 mL acetone; based on Sparling and Fellers, 2009), 0.2 μg/L endosulfan (1.6 mg endosulfan [0.996 purity] added to 2,000 mL acetone; based on Sparling and Fellers, 2009), or a mixture of all of these insecticides (combining 250 mL of each stock solution together); all chemicals were purchased from ChemService, Inc. (West Chester, PA). No treatment received more than 0.25 mL acetone per 1 L water, which is below ASTM limits. We sent samples of each pesticide immediately after dosing and 72 h after initial exposure (before water change) to the U.S. Geological Survey in Sacramento, California, to confirm initial concentrations and to determine how much pesticide remained at the time of the water change (Table 1).

We changed the water of tadpoles every third day, changing Bd-controls first to reduce the likelihood of accidental exposure. Although we did not reapply Bd treatments after initial exposure, we reapplied insecticide treatments at each water change. We fed tadpoles ad libitum TetraMin® Tropical Fish Flakes (47% minimum crude protein) at each water change. We searched for metamorphs daily and removed individuals from beakers at metamorphosis (emergence of at least one forelimb, Gosner Stage 42; Gosner, 1960) and placed them in containers with water until tail resorption. We determined time to metamorphosis, mass at metamorphosis, time of tail resorption, and survival to metamorphosis before using metamorphs in the feeding trial (below). Tadpole activity was measured by a single observation on experimental day 31 (after a water change and feeding). A single observer made a <5-sec observation and recorded whether the tadpole was active (feeding or swimming) or resting; observations were made without knowledge of treatment assignments.

After tail absorption, metamorphs were placed individually in plastic shoebox containers (20 × 31 × 10.5 cm) for 24 h containing two moist paper towels and 20 crickets (10-day-old house crickets, *Acheta domesticus*). After 24 h in the metamorph container, the remaining crickets were counted to determine the number eaten.

To determine whether Treefrog metamorphs were Bd-positive, we swabbed each frog ($N = 190$) on their entire ventral surface using a sterile cotton swab following the feeding trial. Swabs were placed individually in an empty vial and frozen at −70°C. The frog was then euthanized in MS-222 and placed in 200-proof denatured ethanol. These samples were analyzed by qPCR (Boyle et al., 2004) at a U.S. Geological Survey Laboratory (Reston, VA).

TABLE 1. Spiked and measured water concentration (conc) of insecticides immediately after dosing and 72 h later (before water change and renewal of insecticide).

Treatment	Spike conc (μg/L)	Hour	Measured conc (μg/L)
Chlorpyrifos	4	0	3.64
		72	2.18
Endosulfan (I and II)	0.2	0	0.273
		72	0.149
Diazinon	75	0	70.1
		72	52.4
Malathion	30	0	29.4
		72	20.3

We used analysis of variance (ANOVA) to test for difference between water and acetone control treatments in the metamorphic response (described below), survival, and activity. Because there were no significant differences between acetone and water controls ($F_{1,24} \leq 0.24$, $P \geq 0.6301$), we eliminated acetone controls from the subsequent analyses (including or removing acetone controls had no effect on the outcome of the results). We analyzed how Bd-exposure, pesticide exposure, and their interaction affected the "metamorphic response," which consisted of time to metamorphosis (Gosner stage 42), time of tail resorption (time between Gosner stage 42 and 46), mass at metamorphosis (Gosner stage 46), and number of crickets eaten by metamorphs (number eaten/20) using a MANOVA. Significant effects in the MANOVA were followed up with univariate ANOVAs to evaluate which responses were affected by treatments and their interaction. We analyzed the effect of Bd-exposure, pesticide exposure, and their interaction on survival to metamorphosis and tadpole activity using ANOVA; these two factors were not included in the multivariate analysis to prevent replicates with missing cells in some responses (attributable to tadpole mortality) from being eliminated from the analysis.

RESULTS

The presence of Bd decreased survival to metamorphosis by approximately 8%, but survival was not affected significantly by insecticide exposure or the interaction of Bd and insecticide exposure (Table 2; Fig. 1A). Activity of tadpoles was also reduced by 43% in presence of Bd but not by other treatments (Table 2; Fig. 1B). The multivariate metamorphic response was affected by Bd but not by pesticides or treatment interactions (Table 2; Fig. 2). The effect of Bd on the metamorphic response was mainly attributable to effects on time to metamorphosis and time to tail resorption. Bd lengthened the larval period by approximately 2.5 days (Fig. 2A) and slowed the time of tail resorption by 0.5 days (Table 2; Fig. 2B). Mass at metamorphosis was marginally reduced by Bd, although the reduction was relatively small (Table 2; Fig. 2C). The number of crickets eaten by metamorphs did not vary with Bd exposure (Table 2; Fig 2D). All metamorphs, regardless of treatment, tested negative for Bd.

DISCUSSION

Many amphibian population declines and extinctions have been associated with Bd (Berger et al., 1998; Daszak et al., 2003; Lips et al., 2008), and experimental studies have confirmed that Bd in the absence of other factors can negatively affect growth and survival in some amphibians (Parris and Cornelius, 2004; Blaustein et al., 2005; Carey et al., 2006). Some researchers, however, have speculated that Bd alone may not explain population declines and that certain environmental conditions like presence of pesticides could increase vulnerability to pathogens (Parris and Baud, 2004; Hayes et al., 2006; Rohr and Raffel, 2010). Our study, however, suggests that the negative effects of Bd exposure were not exacerbated by the presence of sublethal concentrations of single or mixtures of insecticides at levels measured in nature.

Bd exposure alone had negative effects on survival, tadpole activity, and the metamorphic response (including time to metamorphosis, mass at metamorphosis, and time to tail absorption). Other studies found similar negative effects of Bd on amphibians of other species (Parris and Cornelius, 2004; Garner et al., 2009). For instance, Venesky et al. (2010a) found that Bd infection can reduce tadpole activity. Reduced activity can decrease predation rates (Skelly and Werner, 1990) and may increase the probability of infected individuals acting as a reservoir for other species that enter aquatic environment at later times (although our anurans did not test positive for Bd at metamorphosis). Reduced activity with Bd exposure could also explain increased time to metamorphosis and the marginally reduced mass at metamorphosis observed in our study. Longer larval periods and reduced mass from Bd infection can also result from depigmentation of keratinized tissues and loss of teeth (Rachowicz and Vredenburg, 2004; Knapp and Morgan, 2006; Venesky et al., 2010c) and from altered feeding kinematics, which can reduce foraging efficiency (Venesky et al., 2010a). Impacts on time and size at metamorphosis alone could have long-lasting effects and negatively impact future reproduction and survival (Smith, 1987; Semlitsch et al., 1988; Scott, 1994), even if animals were not infected with Bd at metamorphosis.

It was surprising that all the swabs from Treefrogs tested negative for Bd, because we found negative treatment-level effects and negative trends for most endpoints examined. However, Garner et al. (2009) also found negative effects of Bd on survival and growth, even though individuals were often not infected at the end of the study, as we found. Such results suggest there is a physiological cost to fighting Bd. It is possible that tadpoles were never infected, but if this was the case, it is difficult to explain why the Bd treatment was significantly different from controls (because controls received the same aged tap water that was used to wash Bd-infected plates). Alternatively, it is possible that tadpoles were infected but were able to clear the infection. Initial infection rates may have also been low because of limited keratinized tissues early in development (which would be limited to mouth parts). Additionally, Venesky (2011) found that only 15% of Southern Leopard Frog tadpoles fed high protein (47.6% protein) diets were infected with Bd after exposure, whereas 65% of tadpoles on lower protein (13.8% protein) diets were infected. Our tadpoles were fed a high protein diet (47% protein), which may have increased their resistance. Additionally, Garcia et al. (2006) found that Pacific Treefrogs were less susceptible to Bd compared to Western Toads (*Bufo boreas*) or Cascades Frogs (*Rana cascadae*). Thus, a combination of a high protein diet and a species with a lower susceptibility to Bd may have contributed to the absence of Bd infection.

We anticipated that tadpoles chronically exposed to realistic field concentrations of insecticides (McConnell et al., 1998; Fellers et al., 2007), which represented approximately 1% of reported LC50s (detailed in the Materials and Methods), could have lead to negative effects at metamorphosis (as in Bridges, 2000) and increased incidence of disease (as in Hayes et al.,

TABLE 2. Summary of analyses of variance (ANOVA) for survival to metamorphosis and tadpole activity, and multivariate analysis of variance (MANOVA) for metamorphic response. The MANOVA was followed by univariate ANOVAs to determine which factors were the main contributor to main effects.

Response	Source of variation	df	*F*	*P*
Survival to metamorphosis	Bd	1	4.88	0.0284
	Pesticide	5	0.49	0.7853
	Bd × pesticide	5	0.67	0.6463
	Error	192		
Tadpole activity	Bd	1	4.12	0.0438
	Pesticide	5	0.44	0.8226
	Bd × pesticide	5	0.43	0.8286
	Error	183		
Metamorphic response	Bd	4,170	5.38	0.0004
	Pesticide	20,565	0.79	0.7271
	Bd × pesticide	20,565	0.95	0.5225
Time to metamorphosis	Bd	1	8.34	0.0004
	Pesticide	5	1.22	0.3032
	Bd × pesticide	5	0.85	0.5157
	Error	173		
Time to tail resorption	Bd	1	10.95	0.0011
	Pesticide	5	0.79	0.5570
	Bd × pesticide	5	0.22	0.9528
	Error	173		
Mass at metamorphosis	Bd	1	3.75	0.0544
	Pesticide	5	0.68	0.6396
	Bd × pesticide	5	1.60	0.1614
	Error	173		
Number of crickets eaten	Bd	1	1.35	0.2463
	Pesticide	5	0.53	0.7511
	Bd × pesticide	5	0.98	0.4339
	Error	173		

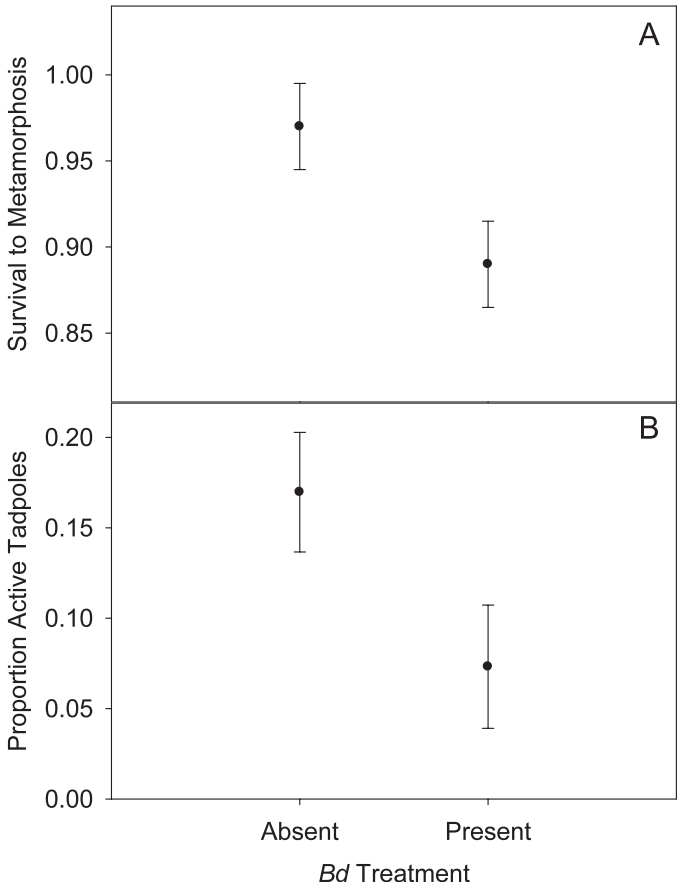

FIG. 1. Survival to metamorphosis (A) and proportion of active tadpoles (B) for individuals reared in the absence or presence of Bd. Error bars represent ± 1 SE.

2006). However, no effects of insecticide exposure were observed. Gahl et al. (2011) examined the effects of Bd on Wood Frog (*Lithobates sylvaticus*) tadpoles in the presence of the herbicide glyphosate and also found that these factors did not interact. If the presence of these insecticides increased susceptibility to disease (or if disease presence increased susceptibility to insecticides), tadpoles exposed to both Bd and insecticide would have shown more negative effects on responses measured. Although our study does not support this hypothesis, the concentrations we used were low (though realistic), and greater concentrations of insecticides may increase susceptibility to Bd or other pathogens.

Our study does not support the hypothesis that relevant environmental concentrations of insecticides alone or in combination can increase susceptibility to Bd or enhance negative effects of Bd. However, our study examined low, but realistic concentrations of insecticides, which may not have affected immunity; in nature, pulses of higher exposures could increase vulnerability, and other contaminants may have different impacts on susceptibility. Evaluating the potential for interaction among pesticides and disease pathogens remains vital because, although disease spread may be difficult to stop or control, limits could be set on pesticide use and application.

Acknowledgments.—We appreciate the assistance of J. Duncan and C. Distel in the lab and P. Kleeman who assisted with the collection of Pacific Treefrog eggs. B. Rothermel, M. Parris, M. Venesky, and F. Brem kindly provided advice on culturing Bd and exposing tadpoles to Bd. We are grateful to M. Fisk, T. Ratliff, and K. Minick who helped us with sterile technique and culturing Bd. Thanks to J. Kirshtein for testing our samples for Bd and K. Smalling for analyzing the exposure concentration of

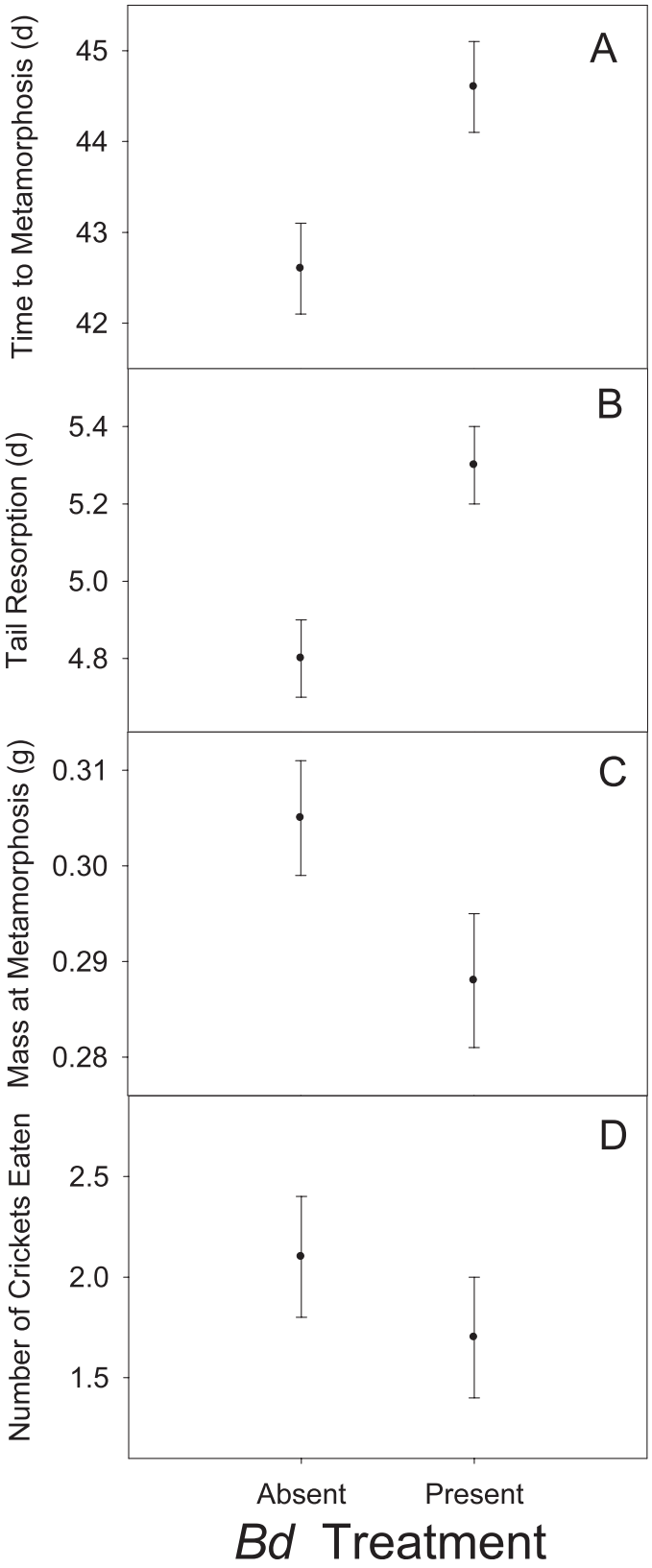

FIG. 2. The metamorphic response of time to metamorphosis (Gosner stage 42); (A), time to tail resorption (B), mass at metamorphosis (C), and number of crickets eaten by metamorphs (D) of tadpoles reared in the absence or presence of Bd. Error bars represent ± 1 SE.

our insecticides. This manuscript was improved by the thoughtful comments of M. Youngquist, S. Rumschlag, and three anonymous reviewers. This research was conducted under IACUC Protocol 740, and funded by Miami University and the U.S. Geological Survey. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. government. This is contribution 413 of the U.S. Geological Survey Amphibian Research and Monitoring Initiative (ARMI).

LITERATURE CITED

ANGERMANN, J. E., G. M. FELLERS, AND F. MATSUMURA. 2002. Polychlorinated biphenyls and toxaphene in Pacific Tree Frog tadpoles (*Hyla regilla*) from the California Sierra Nevada, USA. Environmental Toxicology and Chemistry 21:2209–2215.

BERGER, L., R. SPEARE, P. DASZAK, D. E. GREEN, A. A. CUNNINGHAM, C. L. GOGGIN, R. SLOCOMBE, M. A. RAGAN, A. D. HYATT, K. R. MCDONALD, ET AL. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forest of Australia and Central America. Proceedings of the National Academy of Science USA 95: 9031–9036.

BLAKLEY, B., P. BROUSSEAU, M. FORUNIER, AND I. VOCCIA. 1999. Immunotoxicolgy of pesticides: a review. Toxicology and Industrial Health 15: 119–132.

BLAUSTEIN, A. R., J. M. ROMANSIC, E. A. SCHEESSELE, B. A. HAN, A. P. PESSIER, AND J. E. LONGCORE. 2005. Interspecific variation in susceptibility of frog tadpoles to the pathogenic fungus *Batrachochytrium dendrobatidis*. Conservation Biology 19:1460–1468.

BOONE, M. D., AND C. M. BRIDGES. 2003. Impacts of chemical contaminants on amphibian populations. *In* R. D. Semlitsch (ed.), Amphibian Conservation, pp. 152–167. Smithsonian Press, Washington, DC.

BOONE, M. D., AND R. D. SEMLITSCH. 2001. Interactions of insecticide with larval density and predation in experimental amphibian communities. Conservation Biology 15:228–238.

BOYLE, D. G., D. B. BOYLE, V. OLSEN, J. A. T. MORGAN, AND A. D. HYATT. 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. Diseases of Aquatic Organisms 60:141–148.

BRIDGES, C. M. 2000. Long-term effects of pesticide exposure at various life stages of the Southern Leopard Frog (*Rana sphenocephala*). Archives of Environmental Contamination and Toxicology 39:91–96.

BRIGGS, C. J., V. T. VREDENBURG, R. A. KNAPP, AND L. J. RACHOWICZ. 2005. Investigating the population-level effects of chytridiomycosis: an emerging infectious disease of amphibians. Ecology 86:3149–3159.

CAREY, C., AND C. J. BRYANT. 1995. Possible interrelations among environmental toxicants, amphibian development, and decline of amphibian populations. Environmental Health Perspectives 103:13–14.

CAREY, C., J. E. BRUZGUL, L. J. LIVO, M. L. WALLING, K. A. KUEHL, B. F. DIXON, A. P. PESSIER, R. A. ALFORD, AND K. B. ROGERS. 2006. Experimental exposures of Boreal Toads (*Bufo boreas*) to a pathogenic chytrid fungus (*Batrachochytrium dendrobatidis*). EcoHealth 3:5–21.

CHRISTIN, M. S., A. D. GENDRON, P. BROUSSEAU, L. MENARD, D. J. MARCOGLIESE, D. CYR, S. RUBY, AND M. FOURNIER. 2003. Effects of agricultural pesticides on the immune system of *Rana pipiens* and on its resistance to parasitic infection. Environmental Toxicology and Chemistry 22:1127–1133.

COLLINS, J. P., AND M. L. CRUMP. 2010. Extinction in Our Times: Global Amphibian Decline. Oxford University Press, New York.

CONLON, J. M. 2011. The contribution of skin antimicrobial peptides to the system of innate immunity in anurans. Cell Tissue Research 343: 201–212.

DASZAK, P., A. A. CUNNINGHAM, AND A. D. HYATT. 2003. Infectious disease and amphibian population declines. Diversity and Distributions 9: 141–150.

DASZAK, P., D. E. SCOTT, A. M. KILPATRICK, C. FAGGIONI, J. W. GIBBONS, AND D. PORTER. 2005. Amphibian population declines at Savannah River Site are linked to climate not chytridiomycosis. Ecology 86:3232–3237.

Davidson, C. 2004. Declining downwind: amphibian population declines in California and historical pesticide use. Ecological Applications 14: 1892–1902.

Davidson, C., H. B. Shaffer, and M. R. Jennings. 2001. Declines of the California Red-Legged Frog: climate, UV-B, habitat, and pesticides hypotheses. Ecological Applications 14:464–479.

———. 2002. Spatial tests of the pesticide drift, habitat destruction, UV-B, and climate-change hypotheses for California amphibian declines. Conservation Biology 16:1588–1601.

Davidson, C., M. F. Benard, H. B. Shaffer, J. M. Parker, C. O'Leary, J. M. Conlon, and L. A. Rollins-Smith. 2007. Effects of chytrid and carbaryl exposure on survival, growth, and skin peptide defenses in foothill Yellow-Legged Frogs. Environmental Science and Technology 41:1771–1776.

Distel, C. A., and M. D. Boone. 2010. Effects of aquatic exposure to the insecticide carbaryl are species-specific across life stages and mediated by heterospecific competitors in anurans. Functional Ecology 24:1342–1352.

Fellers, G. M., D. F. Bradford, D. Pratt, and L. L. Wood. 2007. Demise of repatriated populations of mountain Yellow-Legged Frogs (*Rana muscosa*) in the Sierra Nevada of California. Herpetological Conservation and Biology 21:5–21.

Fisher, M. C., T. W. J. Garner, and S. F. Walker. 2009. Global emergence of *Batrachochytrium denderobatidis* and amphibian chytridiomycosis in space, time, and host. Annual Review of Microbiology 63:291–310.

Gahl, M. K., B. D. Pauli, and J. E. Houlahan. 2011. Effects of chytrid fungus and a glyphosate-based herbicide on survival and growth of Wood Frogs (*Lithobates sylvaticus*). Ecological Applications doi:10.1890/10–2319.1.

Garcia, T. S., J. M. Romansic, and A. R. Blaustein. 2006. Survival of three species of anuran metamorphs exposed to UV-B radiation and the pathogenic fungus *Batrachochytrium dendrobatidis*. Disease of Aquatic Organisms 72:163–169.

Garner, T. W., S. Walker, J. Bosch, S. Leech, J. M. Rowcliffe, A. A. Cunningham, and M. C. Fisher. 2009. Life history tradeoffs influence mortality associated with the amphibian pathogen *Batrachochytrium dendrobatidis*. Oikos 118:783–791.

Gendron, A. D., D. J. Marcogliese, S. Barbeau, A. S. Christin, P. Brousseau, S. Ruby, D. Cyr, and M. Fournier. 2003. Exposure of Leopard Frogs to a pesticide mixture affects life history characteristics of the lungworm *Rhabdias ranae*. Oecologia 135:469–476.

Gosner, N. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16:183–190.

Harris, R. N., T. Y. James, A. Lauer, M. A. Simon, and A. Patel. 2006. Amphibian pathogen *Batrachochytrium dendrobatidis* is inhibited by the cutaneous bacteria of amphibian species. EcoHealth 3:53–56.

Hayes, T. B., P. Case, C. Chui, D. Chung, C. Haeffele, K. Haston, M. Lee, V. P. Mai, Y. Marjuoa, J. Parker, et al. 2006. Pesticide mixtures, endocrine disruption, and amphibian declines: are we underestimating the impact? Environmental Health Perspectives 114:40–50.

Hayes, T. B., P. Falso, S. Gallipeau, and M. Stice. 2010. The cause of global amphibian declines: a developmental endocrinologist's perspective. Journal of Experimental Biology 213:921–933.

Houlahan, J. E., C. S. Findlay, B. R. Schmidt, A. H. Meyer, and S. L. Kuzmin. 2000. Quantitative evidence for global amphibian population declines. Nature 404:752–755.

Knapp, R. A., and J. A. T. Morgan. 2006. Tadpole mouthpart depigmentation as an accurate indicator of chytridiomycosis, an emerging disease of amphibians. Copeia 2006:188–197.

Kuivila, K. M., and C. G. Foe. 1995. Concentrations, transport, and biological effects of dormant spray pesticides in the San Francisco Estuary, California. Environmental Toxicology and Chemistry 14: 1141–1150.

Lips, K. R., J. Diffendorfer, J. R. Mendelson III, and M. W. Sears. 2008. Riding the wave: reconciling the roles of disease and climate change in amphibian declines. PLoS Biology 6:441–454.

Longcore, J. R., J. E. Longcore, A. P. Pessier, and W. A. Halteman. 2007. Chytridiomycosis widespread in anurans of northeastern United States. Journal of Wildlife Management 71:435–444.

McConnell, L. L., J. S. LeNoir, S. Datta, and J. N. Seiber. 1998. Wet deposition of current-use pesticides in the Sierra Nevada mountain range, California, USA. Environmental Toxicology and Chemistry 17:1908–1916.

Mills, N. E., and R. D. Semlitsch. 2004. Competition and predation mediate indirect effects of an insecticide on Southern Leopard Frogs. Ecological Applications 14:1041–1054.

Muths, E., P. S. Corn, A. P. Pessier, and D. E. Green. 2003. Evidence for disease-related amphibian decline in Colorado. Biological Conservation 110:357–365.

Parris, M. J., and D. R. Baud. 2004. Interactive effects of a heavy metal and chytridiomycosis on Gray Treefrog larvae (*Hyla chrysoscelis*). Copeia 2004:344–350.

Parris, M. J., and T. O. Cornelius. 2004. Fungal pathogen causes competitive and developmental stress in larval amphibian communities. Ecology 85:3385–3395.

Rachowicz, L. J., and V. T. Vredenburg. 2004. Transmission of *Batrachochytrium dendrobatidis* within and between amphibian life stages. Diseases of Aquatic Organisms 61:75–83.

Relyea, R. A. 2003. Predator cues and pesticides: a double dose of danger for amphibians. Ecological Applications 13:1515–1521.

———. 2004. Synergistic impacts of malathion and predatory stress on six species of North American tadpoles. Environmental Toxicology and Chemistry 23:1080–1084.

———. 2009. A cocktail of contaminants: how mixtures of pesticides at low concentrations affect aquatic communities. Oecologia 159:363–376.

Relyea, R. A., and J. Hoverman. 2006. Assessing the ecology in ecotoxicology: a review and synthesis in freshwater systems. Ecology Letters 9:1157–1171.

Relyea, R. A., and N. M. Mills. 2001. Predator-induced stress makes the pesticide carbaryl more deadly to Gray Treefrog tadpoles (*Hyla versicolor*). Proceedings of the National Academy of Sciences USA 98: 2491–2496.

Rohr, J. R., and T. R. Raffel. 2010. Linking global climate and temperature variability to widespread amphibian declines putatively caused by disease. Proceedings of the National Academy of Sciences USA 107:8269–8274.

Romansic, J. M., P. T. J. Johnson, C. L. Searle, J. E. Johnson, T. S. Tunstall, B. A. Han, J. R. Rohr, and A. R. Blaustein. 2011. Individual and combined effects of multiple pathogens on Pacific Treefrogs. Oecologia 166:1029–1041.

Rothermel, B. B., S. C. Walls, J. C. Mitchell, C. K. Dodd, L. K. Irwin, D. E. Green, V. M. Vazquez, J. W. Petranka, and D. J. Stevenson. 2008. Widespread occurrence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* in the southeastern USA. Diseases of Aquatic Organisms 82:3–18.

Scott, D. E. 1994. The effect of larval density on adult demographic traits in *Ambystoma opacum*. Ecology 75:1383–1396.

Semlitsch, R. D., D. E. Scott, and J. H. K. Pechmann. 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. Ecology 69:184–192.

Skelly, D. K., and E. E. Werner. 1990. Behavioral and life-historical responses of larval American Toads to an odonate predator. Ecology 71:2313–2322.

Skerratt, L. F., L. Berger, R. Speare, S. Cashins, K. R. McDonald, A. D. Phillott, H. B. Hines, and N. Kenyon. 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. EcoHealth 4:125–134.

Smith, D. C. 1987. Adult recruitment in Chorus Frogs: effects of size and date at metamorphosis. Ecology 68:344–350.

Sparling, D. W., and G. M. Fellers. 2007. Comparative toxicity of chlorpyrifos, diazinon, malathion, and their oxon derivatives to larval *Rana boylii*. Environmental Pollution 147:535–539.

———. 2009. Toxicity of two insecticides to California, USA, anurans and its relevance to declining amphibian populations. Environmental Toxicology and Chemistry 28:1696–1703.

Sparling, D. W., G. M. Fellers, and L. L. McConnell. 2001. Pesticides and amphibian population declines in California, USA. Environmental Toxicology and Chemistry 20:1591–1595.

Stockwell, M. P., J. Clulow, and M. J. Mahony. 2010. Host species determines whether infection load increases beyond disease-causing thresholds following exposure to the amphibian chytrid fungus. Animal Conservation 13:62–71.

Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D. Fischman, and R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306:1783–1786.

Taylor, S. K., E. S. Williams, and K. W. Mills. 1999. Effects of malathion on disease susceptibility in Woodhouse's Toads. Journal of Wildlife Diseases 25:536–541.

Tobler, U., and B. R. Schmidt. 2010. Within- and among-population variation in chytridiomycosis-induced mortality in the toad *Alytes obstetricans*. PLos One 5:e10927.

VENESKY, M. D. 2011. Dynamics of an Emerging Infectious Disease of Amphibians: From Individuals to Communities. Unpubl. Ph.D. diss., University of Memphis, Memphis, TN.

VENESKY, M. D., M. J. PARRIS, AND A. STORFER. 2010a. Impacts of *Batrachochytrium dendrobatidis* infection on tadpole foraging performance. EcoHealth 6:565–575.

VENESKY, M. D., R. J. WASSERSUG, AND M. J. PARRIS. 2010b. Fungal pathogen changes the feeding kinematics of larval anurans. Journal of Parasitology 96:552–557.

———. 2010c. How does change in labial tooth row number affect feeding kinematics and foraging performance of a ranid tadpole (*Lithobates sphenocehalus*)? Biology Bulletin 218:160–168.

VREDENBURG, V. T., R. A. KNAPP, T. S. TUNSTALL, AND C. J. BRIGGS. 2010. Dynamics of an emerging disease drive large-scale amphibian population extinction. Proceedings of the National Academy of Sciences USA 107:9689–9694.

WIDDER, P. D., AND J. R. BIDWELL. 2008. Tadpole size, cholinesterase activity, and swim speed in four frog species after exposure to sublethal concentrations of chlorpyrifos. Aquatic Toxicology 88: 9–18.

WOODHAMS, D. C., K. ARDIPRADJA, R. A. ALFORD, G. MARANTELLI, L. K. REINERT, AND L. A. ROLLINS-SMITH. 2007. Resistance to chytridiomycosis varies among amphibian species and is correlated with skin peptide defenses. Animal Conservation 10:409–417.

Accepted: 17 October 2011.