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Effects of chytrid fungus and a glyphosate-based herbicide on survival and growth of wood frogs (*Lithobates sylvaticus*)

Megan K. Gahl,[1,3] Bruce D. Pauli,[2] and Jeff E. Houlahan[1]

[1]*Department of Biology, Canadian Rivers Institute, University of New Brunswick, P.O. Box 5050, Saint John, New Brunswick E2L 4L5 Canada*
[2]*Environment Canada, National Wildlife Research Centre, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario K1A OH3 Canada*

Abstract. Anthropogenic-derived stressors in the environment, such as contaminants, are increasingly considered important cofactors that may decrease the immune response of amphibians to pathogens. Few studies, however, have integrated amphibian disease and contaminants to test this multiple-stressor hypothesis for amphibian declines. We examined whether exposure to sublethal concentrations of a glyphosate-based herbicide and two strains of the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*) could: (1) sublethally affect wood frogs (*Lithobates sylvaticus*) by altering the time to and size at metamorphosis, and (2) directly affect survivability of wood frogs after metamorphosis. Neither *Bd* strain nor herbicide exposure alone significantly altered growth or time to metamorphosis. The two *Bd* strains did not differ in their pathogenicity, and both caused mortality in post-metamorphic wood frogs. There was no evidence of an interaction between treatments, indicating a lack of herbicide-induced susceptibility to *Bd*. However, the trends in our data suggest that exposure of wood frogs to a high concentration of glyphosate-based herbicide may reduce *Bd*-caused mortality compared to animals exposed to *Bd* alone. These results exemplify the complexities inherent when populations are coping with multiple stressors. In this case, the perceived stressor, glyphosate-based herbicide, appeared to affect the pathogen more than the host's immune system, relieving the host from disease-caused effects. This suggests caution when invoking multiple stressors as a cause for increased disease susceptibility and indicates that the effects of multiple stressors on disease outcome depend on the interrelationships of stressors to both the pathogen and the host.

Key words: amphibian disease; Batrachochytrium dendrobatidis; *chytrid fungus; chytridiomycosis; cofactors; glyphosate; herbicide; immune response;* Lithobates sylvaticus; *Long-term Experimental Wetlands Area, southwestern New Brunswick, Canada; multiple stressors; wood frog.*

Introduction

Amphibian populations are declining around the world and amphibian disease is often implicated in population declines (Houlahan et al. 2000, Stuart et al. 2004). Anthropogenic stressors in the environment, such as agrochemicals, are increasingly considered important cofactors that may decrease the immune response of amphibians to disease and thereby increase amphibian disease prevalence (Carey et al. 1999, Forson and Storfer 2006). However, relatively few studies have examined the interactions of contaminants and amphibian disease. Those that have included interactions have focused on the effects of insecticides (Christin et al. 2003, Davidson et al. 2007) and on parasites (Gendron et al. 2003, Rohr et al. 2008), whereas the sublethal effects of other widely used agrochemicals, such as herbicides, and other non-parasite pathogens are largely understudied (but see Forson and Storfer 2006).

Glyphosate-based herbicides are widely used herbicides (Woodburn 2000) with both forestry and agricultural applications. In both types of application, herbicide may reach nearby small water bodies that house breeding populations of amphibians. Lethal and sublethal effects of glyphosate-based herbicides on amphibians have been demonstrated in experimental settings in both the field and laboratory (Chen et al. 2004, Howe et al. 2004, Relyea 2005, Comstock et al. 2007, Relyea and Jones 2009). However, lethal concentrations in experimental settings are often several orders of magnitude higher than levels of glyphosate-based herbicides measured in the environment (Giesy et al. 2000, Thompson et al. 2004, Struger et al. 2008, Battaglin et al. 2009). Therefore, if glyphosate-based herbicides are a factor in amphibian declines in free-living populations, it may be through sublethal effects and interactions with other stressors.

Chytridiomycosis, caused by the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), is an important

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[3] E-mail: mkgahl@bates.edu

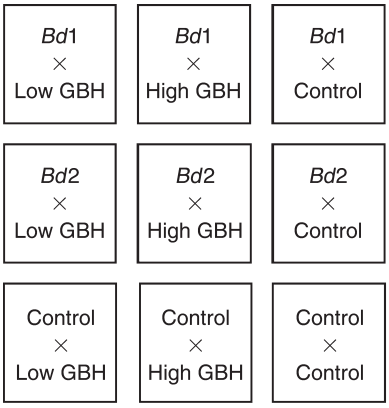

FIG. 1. Nine treatments for wood frogs (*Lithobates sylvaticus*). The experimental design was a full 3 × 3 cross of two glyphosate-based herbicide concentrations (low and high GBH) and a control with two strains of the pathogenic chytrid fungus *Bd* (*Batrachochytrium dendrobatidis*) and a control. Each treatment box represents 10 aquaria.

amphibian disease linked to amphibian population declines and extinctions around the world (Daszak et al. 1999, Kilpatrick et al. 2009). However, lethality caused by *Bd* varies among amphibian species (Blaustein et al. 2005, Woodhams et al. 2007). In addition, different *Bd* strains may vary in their virulence to amphibians (Berger et al. 2005, Retallick and Miera 2007, Fisher et al. 2009). Because this fungus feeds on keratin, which is found in the skin of post-metamorphic amphibians, *Bd* primarily affects post-metamorphic and adult amphibians. Amphibian larvae can be infected, but are often not killed by *Bd* at this life stage because they have keratin only in their tooth rows and jaw sheaths (but see Blaustein et al. 2005). Therefore, some amphibian species or life stages may be infected, but not affected, by *Bd*.

Two hypotheses for the recent spread of *Bd*-caused amphibian mortalities have been promoted (Rachowicz et al. 2005). The first proposes that *Bd* is an emerging disease, transported around the world with human movement, that causes mortalities in naïve populations of amphibians (Morehouse et al. 2003, Morgan et al. 2007, Picco and Collins 2008, Schloegel et al. 2009). The alternative hypothesis is that *Bd* is not necessarily novel in many areas, but rather that sublethal stressors act synergistically with *Bd* infection to cause increased mortalities (e.g., Carey 1993), as has been demonstrated with some amphibian parasites (Kiesecker 2002, Rohr et al. 2008). This latter theory may be particularly important for species that seem to be largely unaffected by *Bd* in the wild. For example, wood frogs [*Lithobates sylvaticus* (LeConte, 1825), formerly *Rana sylvatica*; see Plate 1] are widely distributed across North America (Hunter et al. 1999). *Bd* has been identified in wild-caught adult wood frogs (Rittman et al. 2003, Green and Muths 2005, Longcore et al. 2007), but large-scale declines of wood frog populations caused by *Bd* have not been documented. Whether wood frogs are susceptible to *Bd* and whether a synergistic effect of a sublethal stressor can induce stronger pathogenic effects of *Bd* is unclear.

In this study we examined the effects of glyphosate-based herbicide exposure on the pathogenic effects of two strains of *Bd* using wood frogs as our experimental model. Wood frogs breed in small, temporary ponds typically associated with forest or agricultural settings where glyphosate-based herbicides may be applied (Thompson et al. 2004, Battaglin et al. 2009). We conducted laboratory experiments to investigate whether sublethal doses of a widely used glyphosate-based herbicide affect wood frog susceptibility to *Bd* in both larval and early post-metamorphic life stages. Specifically, we examined how exposure to glyphosate-based herbicides and *Bd* may: (1) sublethally affect wood frog larvae by altering time to and size at metamorphosis, and (2) directly affect survivability of wood frogs after metamorphosis.

METHODS

Amphibian collection and handling

We collected wood frog embryos from forested breeding wetlands in April 2009 at the Long-term Experimental Wetlands Area (LEWA) in southwestern New Brunswick, Canada. We obtained a genetically diverse sample of eggs from the study site by using embryos from over 1300 egg masses that were moved around LEWA as part of another study. Prior to April 2009, there were no glyphosate applications in the egg collection areas. Eggs were hatched in a single large glass aquarium in the laboratory and hatchlings were haphazardly selected and assigned to treatment tanks. We used 10 aquaria for each treatment, in a full 3 × 3 cross with two glyphosate concentrations with a control and two *Bd* strains with a control, resulting in nine treatments and 90 aquaria in total (Fig. 1). Each aquarium initially housed six wood frog larvae. Amphibians were kept at room temperature (17–22°C) throughout the study, corresponding with ideal growth temperatures for *Bd* (Piotrowski et al. 2004).

To house larvae, we used 6.6-L plastic aquaria (2007 Sterilite Corporation, Townsend, Massachusetts, USA) drilled with 1/8-inch (0.318-cm) holes along the upper edge. We filled each aquarium with 4 L of dechlorinated freshwater and maintained this volume until wood frogs completed metamorphosis. Larvae were fed rabbit chow (ZuPreem Nature's Promise Premium Rabbit Food, Premium Nutritional Products, Shawnee, Kansas, USA) 2–3 times per week ad libitum, with leftover food removed prior to the next feeding. Full water changes were performed weekly, except for just after herbicide exposure, when tanks were left undisturbed for 10 days, aside from feeding and *Bd* exposures. Containers were checked for metamorphs and mortalities daily.

Wood frogs began metamorphosing as early as 20 days after herbicide exposure. When at least one larva in

an aquarium began metamorphosis (reaching Gosner stage 42 (Gosner 1960) the water was emptied, the bottom of the aquarium was lined with an unbleached, moist paper towel, and the aquarium was covered with a lid also drilled with holes. Wood frogs remaining as larvae were moved to a smaller, water-filled 300-mL translucent plastic cup (Dorfin, Montreal, Quebec, Canada) placed within the larger aquarium. Paper towels were replaced weekly and water in the smaller larva containers was changed twice a week. Metamorphs were fed pinhead crickets or wingless fruit flies 2–3 times per week ad libitum. Aquaria were checked for mortalities daily.

Wood frog larvae were staged (following Gosner 1960) at the beginning of exposures, and were measured and weighed at the onset of metamorphosis (Gosner stage 42). Animals that died during the experiment were preserved in 70% ethanol for later examination of *Bd* infection. All remaining animals were euthanized on 10 August 2009.

Glyphosate-based herbicide exposure

We exposed wood frog larvae to a glyphosate-based herbicide (Roundup WeatherMAX Herbicide, Monsanto Canada, Winnipeg, Manitoba, Canada) on 8 June 2009, when larvae were at a Gosner stage of 31.7 ± 2.0 (mean ± SD), that is, when the foot paddle is distinguishable, but toes are not yet so (Gosner 1960). We used two levels of herbicide exposure and a control: 0.21 mg glyphosate acid equivalents (a.e.)/L (low), and 2.89 mg a.e./L (high). The low concentration was based on concentrations observed in surface waters (Struger et al. 2008). The high concentration was based on an expected concentration following an application at the maximum label rate into 15 cm of water with no intercepting vegetation, and corresponds with published lethal concentrations for wood frogs to another glyphosate-based herbicide, Roundup Original Max (Relyea and Jones 2009). Because of the small amount of herbicide added to each aquarium, we diluted 0.7 mL prepared herbicide with 1 L dechlorinated freshwater and added 31 mL of this mixture directly to each of the high-herbicide aquaria. We then diluted 70 mL of the remaining solution with an additional 890 mL of water and added 32 mL of this to the low-herbicide aquaria. Following its addition, the herbicide remained in the aquaria and was allowed to naturally degrade until 10 days post-exposure, when we completed full water changes for all aquaria.

Pathogen culture and exposures

We transferred *Bd* cultures from previously cryopreserved agar plates thawed on 25 March 2009, to minimize the amount of passage in pure culture, which can make strains less virulent (Kilpatrick et al. 2009). We used two strains of *Bd* in exposures of larval wood frogs to examine differences in strain virulence (Berger et al. 2005, Retallick and Miera 2007, Fisher et al. 2009). The first strain (JEL404) was a local North American strain isolated from an apparently healthy *Lithobates catesbeianus* larva collected from Crocker Pond, Maine, USA on 16 June 2004 and cryopreserved on 31 October 2005. The second strain (JEL423) was isolated from larval *Phyllomedusa lemur* collected at El Copé, Panama on 14 December 2004 from a large mortality event; this strain was cryopreserved on 5 July 2005. Both strains were supplied by Dr. Joyce Longcore, University of Maine, Orono, Maine, USA.

Stock *Bd* cultures were maintained in 1% tryptone broth in 250-mL flasks. Stock cultures were made from previously cryopreserved agar plates on 9 April 2009 and were kept at 20°C until growth was evident. On 23 April 2009, stocks were moved into a refrigerator and remained at 4°C until inoculant preparation. On 23 May 2009, we transferred cultures into sterilized 50-mL centrifuge tubes filled with 1% tryptone broth to prepare inoculants. We made 24 tube cultures for each strain. We also sterilized another set of 24 tubes containing blank tryptone to act as controls. Tube cultures were kept at 20°C until exposures.

We prepared inoculants from tube cultures on 10 June 2009. In many studies, antibiotics are added to broth-based inoculants to prevent increased bacterial growth resulting from the high nutrient content of tryptone broth. To avoid adding an additional factor, such as antibiotics, to inoculants, we centrifuged tube cultures at 9000 rpm (87 919 m/s^2) for 30 minutes at 10°C and decanted off the supernatant broth. We re-suspended the solids (zoospores and zoosporangia) in each tube in sterilized water to a volume of 25 mL. We combined all tube cultures for each strain into a single composite sample and counted zoospores and zoosporangia using a hemocytometer. For control inoculant preparation, we decanted off broth from blank culture tubes, refilled each tube with sterilized water to 25 mL, and used the composite sterilized water from the tubes.

We exposed wood frog larvae to *Bd* on 10 June 2009, two days after the glyphosate was added to the aquaria, because we designed the study to examine whether exposure to a contaminant, in this case a glyphosate-based herbicide, would make larvae more susceptible to *Bd*. Larvae from each aquarium were placed in two 236-mL exposure containers (Ziploc Snap-Seal, S. C. Johnson and Son), with three larvae per container, each with 90 mL of ambient aquaria water. Because we used ambient aquaria water to expose larvae, the *Bd* was exposed to any remaining glyphosate in the water. We added 10 mL of inoculum to each exposure container, equivalent to 10^5–10^6 zoosporangia plus 10^7 zoospores for both strains. Larvae were left in exposure containers for 24 h. After 24 h, larvae were returned to aquaria with the water and inoculum from the exposure container.

At the end of the experiment, or when an animal died, fresh carcasses were fixed in 70% ethanol and examined microscopically for *Bd* infection. Toe webbing and skin sloughs were wet mounted with distilled water and the

TABLE 1. Two-factor ANOVA results for effects of herbicide concentration and the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) on response variables of wood frogs (*Lithobates sylvaticus*).

Wood frog variables	df	Mean square	*F*	*P*
Bd				
Snout–vent length	2, 81	0.001	0.44	0.646
Mass at metamorphosis	2, 81	0.001	0.61	0.549
Days until metamorphosis	2, 81	54.2	4.64	0.012
Mortalities	2, 81	0.712	24.15	<0.001
Glyphosate herbicide				
Snout–vent length	2, 81	0.006	2.94	0.059
Mass at metamorphosis	2, 81	0.009	3.94	0.023
Days until metamorphosis	2, 81	55.3	4.74	0.011
Mortalities	2, 81	0.203	6.72	0.002
Bd × Glyphosate				
Snout–vent length	4, 81	0.001	0.65	0.629
Mass at metamorphosis	4, 81	0.001	0.47	0.760
Days until metamorphosis	4, 81	2.8	0.24	0.915
Mortalities	4, 81	0.04	1.35	0.258

Notes: Fixed predictor variables were herbicide concentration (control, low, high) and *Bd* strain (control, JEL404, JEL423); response variables were mean snout–vent length, mean mass at metamorphosis, mean time to metamorphosis, and mortalities per aquarium.

epidermal surface examined at 100–400× for *Bd* thalli. We microscopically examined at least 10 metamorphs from each treatment.

Analysis

We analyzed the following response variables using a two-factor analysis of variance: time to metamorphosis, snout–vent length at metamorphosis, mass at metamorphosis, and post-metamorphic mortality. We used a factorial design and fixed predictor variables of herbicide concentration (control, low, high) and *Bd* strain (control, JEL404, JEL423). We tested for three types of effects: (1) the effects of herbicide concentration, (2) the effects of *Bd* strain, and (3) the interaction of the two predictor variables. Homogeneity of variance was confirmed with boxplots, residual plots, and Levene's test of equality of error variance. We verified normality using stem and leaf plots, normal probability plots, and Kolmogorov-Smirnov tests of normality, but did not transform the data because they were only slightly skewed and had similar variances. We used Bonferroni adjustments for post hoc comparisons of group means. All analyses were performed with SPSS Statistics 17.0 (SPSS, Chicago, Illinois, USA).

RESULTS

Bd and glyphosate-based herbicide showed small or no sublethal effects on wood frog larval development and there was no evidence of an interaction between the two treatments (Table 1). Small, but statistically significant, differences were demonstrated in mass at metamorphosis between low and high glyphosate treatments (high treatment animals were larger), in days to metamorphosis between high glyphosate and both low and control treatments (high treatment animals took longer to reach metamorphosis), and in days to metamorphosis between the two *Bd* strains (animals exposed to JEL423 took longer to metamorphose; Table 2).

However, we did find statistically significant effects of both *Bd* and glyphosate-based herbicides on wood frog mortality after metamorphosis. Both strains of *Bd* produced mortalities in post-metamorphic wood frogs, but the strains did not differ significantly in their lethality (Table 1). Exposure to our high concentration of glyphosate-based herbicide and *Bd* resulted in a trend toward lower mortality compared to animals exposed to *Bd* alone (Fig. 2).

DISCUSSION

In this study, acute exposure to a contaminant did not cause increased vulnerability to disease. The lack of herbicide-induced susceptibility in this study is consistent with findings by Davidson et al. (2007), who found no interaction between exposure to *Bd* and the insecticide carbaryl in foothill yellow-legged frogs (*Rana boylii*). These results challenge the multiple stressors hypothesis for amphibian population declines that postulate that immune function is decreased after contaminant exposure making individuals more susceptible to disease, at least for the *Bd* pathogen.

We documented no larval mortality from glyphosate-based herbicide exposure alone, contrary to some published literature (e.g., Relyea and Jones 2009). This could reflect the fact that we used an acute exposure (i.e., glyphosate concentrations were not replaced over time) to replicate what might happen in a forestry or agricultural application with a single spray period. We also used a different product, WeatherMAX, rather than Roundup Original Max that has been used in previous studies. In addition, Relyea and Jones (2009) exposed larvae at Gosner stage 25, while we exposed them at Gosner stage 32. This is consistent with increasing evidence that developmental stage at expo-

TABLE 2. Wood frog endpoint variables (mean ± SD) for the nine treatments in this study: control, low, and high concentrations of glyphosate-based herbicide (GBH) and control, JEL404, and JEL423 *Bd* strains.

Wood frog variables	Control	Low GBH	High GBH
Control			
Snout–vent length (cm)	1.56 ± 0.06	1.54 ± 0.04	1.59 ± 0.05
Mass at metamorphosis (g)	0.46 ± 0.09	0.44 ± 0.06	0.49 ± 0.04
Days until metamorphosis	50.5 ± 3.6	50.0 ± 1.8	52.0 ± 4.9
Mortalities	0.17 ± 0.17	0.12 ± 0.15	0.10 ± 0.12
JEL404			
Snout–vent length (cm)	1.57 ± 0.05	1.57 ± 0.04	1.59 ± 0.05
Mass at metamorphosis (g)	0.45 ± 0.03	0.43 ± 0.04	0.47 ± 0.04
Days until metamorphosis	48.0 ± 2.4	48.8 ± 3.0	51.0 ± 3.4
Mortalities	0.51 ± 0.14	0.44 ± 0.25	0.27 ± 0.14
JEL423			
Snout–vent length (cm)	1.57 ± 0.05	1.57 ± 0.04	1.58 ±0.02
Mass at metamorphosis (g)	0.44 ± 0.04	0.45 ± 0.04	0.47 ± 0.02
Days until metamorphosis	51.2 ± 2.6	51.0 ± 5.2	54.0 ± 2.4
Mortalities	0.42 ± 0.18	0.49 ± 0.21	0.28 ± 0.15

Note: Endpoints include snout–vent length at metamorphosis, mass at metamorphosis, days until metamorphosis (mean per aquarium), and mortalities (mean proportion per aquarium).

sure is an important predictor of the effects of insecticides and herbicides on larval amphibians (e.g., Howe et al. 2004).

In contrast with studies that demonstrate lethality after exposure to glyphosate-based herbicide, exposure to high (but sublethal) concentrations of glyphosate-based herbicide in this study decreased post-metamorphic mortality even when overall mortality increased fourfold due to *Bd* exposure. Mortality rates in *Bd*-exposed frogs dropped by approximately half when *Bd* exposure was combined with exposure to high concentrations of glyphosate-based herbicide. We have identified three plausible explanations for the decline in mortality associated with high herbicide exposure found in this study.

First, glyphosate-based herbicide could have a deleterious effect on *Bd* in water, making *Bd* less effective at infecting larval frogs. This finding could be an artifact of our exposure method, in which we exposed animals to glyphosate-based herbicide first and then used that water as part of the inoculant. In this case, the *Bd* zoospores and zoosporangia were exposed to glyphosate-based herbicide as well. In the laboratory we would expect the glyphosate to have a half-life of 7–14 days, although in the field this could be shorter because glyphosate can sorb quickly to sediments (Giesy et al. 2000). Because of the quick disappearance of glyphosate in the environment, the main interaction in the wild would be any lasting effects of glyphosate-based herbicides or components of the herbicide formulations on frog immune systems. We have tried to replicate this in our experimental design by exposing larvae first to a glyphosate-based herbicide, then to *Bd*, but this design may have had unintended consequences on *Bd*, highlighting the inherent difficulty in choosing an experimental design that best represents the natural world. If the herbicide formulation used in this study has negative impacts on *Bd*, then we would have expected reduced pathogenicity, which was the pattern in our study although it was not statistically significant (Table 1, Fig. 2). Ultimately, we are unable to assess the strength of this explanation with our data because we have no direct assessment of the effects of our glyphosate-based herbicide formulation on *Bd*.

Second, herbicide additions at sublethal concentrations in effect provide additional nutrients to the aquaria that increase the overall fitness of larval amphibians and this increased fitness results in lower post-metamorphic mortality. There is some evidence for this in our data: amphibian larvae exposed to high glyphosate-based herbicide concentrations were heavier at metamorphosis and exhibited increased survival, which was consistent across *Bd* treatments. Previous studies have demonstrated that exposure to glyphosate-based herbicides can cause increased growth in wood frogs (Wojtaszek et al. 2004), but the mechanism was not clear. In this study, the size differences were small (3–4%) and would seem unlikely to result in dramatic differences in post-metamorphic survival. In addition, these wood frog larvae also took longer to reach metamorphosis, so the size difference may be, at least in part, a result of 2–3 extra days of growth rather than increased growth rates. Further, there were no statistically significant differences in mass at metamorphosis for larvae exposed to different levels of glyphosate-based herbicide, but there was a significant difference in post-metamorphic mortality between those treatments (Table 1, Fig. 2). Overall, the evidence for this explanation in this study is weak.

Third, exposure to the glyphosate-based herbicide may stimulate the amphibian immune system and provide increased resistance to disease. There is considerable evidence that variability in immune system response can lead to different infection rates (Richmond et al. 2009). Although most of the current evidence suggests that pesticide exposure has negative impacts on the immune system (Langerveld et al. 2009,

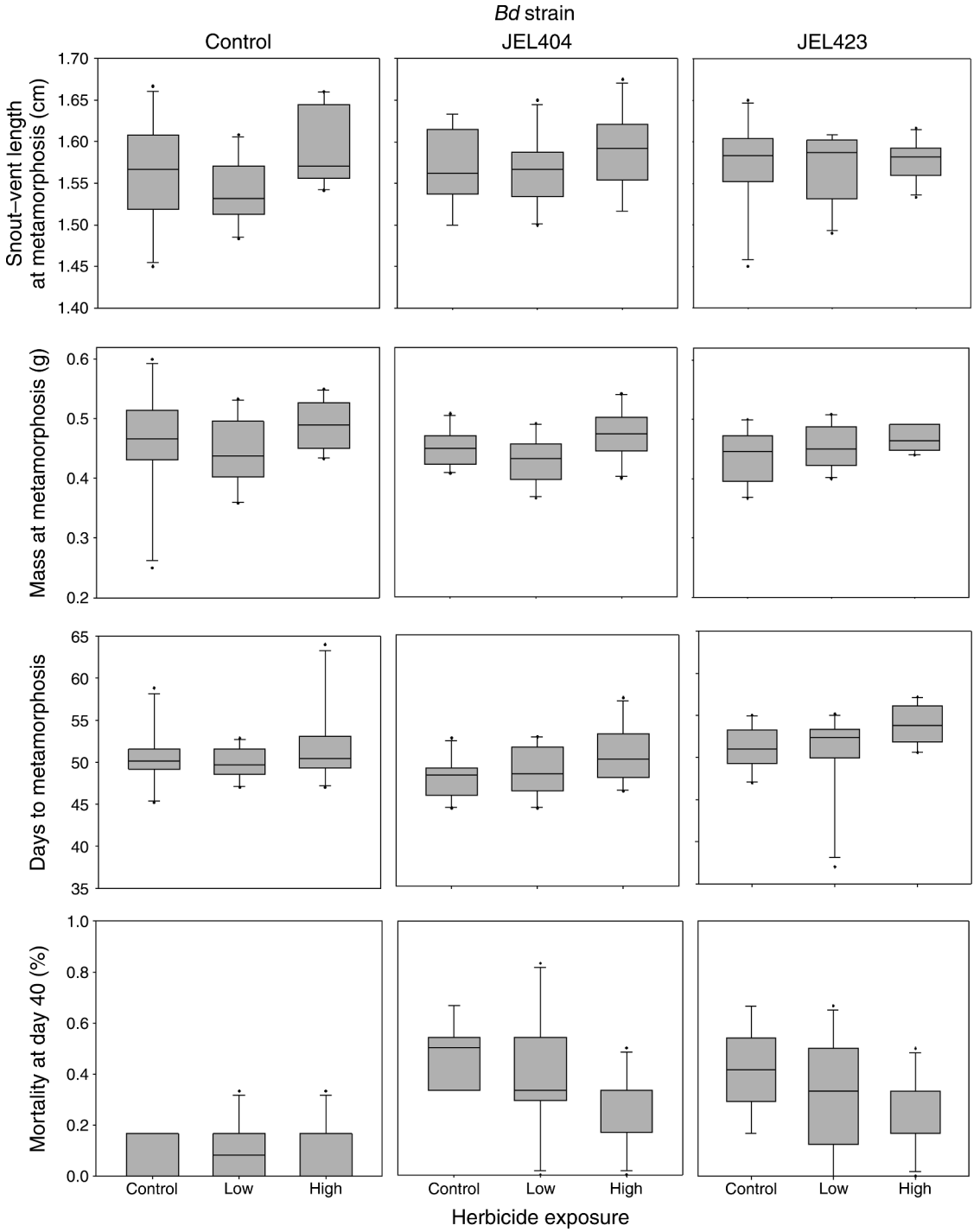

FIG. 2. Snout–vent length and mass at metamorphosis, time to metamorphosis, and mortality of wood frogs exposed to nine treatments including two concentrations of glyphosate-based herbicide (with a control) combined with two *Batrachochytrium dendrobatidis* (*Bd*) strains (with a control), shown as median mean per aquarium. Each box-and-whisker plot summarizes the results from a single treatment. The central line represents the median, the box the interquartile range, the lines the range of measurements, and the dots the suspected outliers.

PLATE 1. Recently metamorphosed wood frog (*Lithobates sylvaticus*) juvenile. Photo credit: M. K. Gahl.

Hayes et al. 2010), there is indication that pesticide exposure can stimulate some aspects of the ranid immune system (Gilbertson et al. 2003). However, the effects of glyphosate-based herbicides on ranid immune systems remain understudied (Paetow 2010). The fact that herbicide exposure resulted in longer time to metamorphosis does suggest that exposure is having effects at the hormonal level. However, the connection between general changes in hormone levels and increased immunity to disease is tenuous at best, so we have little evidence to assess the strength of this explanation with our data. What is clear from our study is that there is no evidence that increased susceptibility to disease is a sublethal effect of acute glyphosate-based herbicide exposure.

We also demonstrated that post-metamorphic wood frogs can die if they are infected with *Bd*, which has implications across the wood frog's range. The significant mortality caused by *Bd* exposure in this study may also be due to the developmental stage at which the wood frogs were exposed, because recent metamorphs are typically more susceptible to *Bd* than later post-metamorphic stages (Lamirande and Nichols 2002). Our data also suggest that environmental conditions may play a role in influencing the mortality induced by *Bd* in free-living populations. For instance, we maintained ideal *Bd* growing conditions of 17–21°C and neutral pH, which are not necessarily conditions that wood frogs are found in across their range (Hunter et al. 1999), perhaps one reason why declines in wood frog populations or mass mortalities caused by *Bd* have not been reported.

Not all wood frogs died following exposure to *Bd* in this study, suggesting that some individuals may be able to carry the pathogen, but not be greatly affected. This is similar to what has been reported for other northeastern North American amphibian species such as bullfrogs (*Lithobates catesbeianus* (Shaw, 1802)) and northern leopard frogs (*Lithobates pipiens*, (Schreber, 1782)) (Daszak et al. 2004, Woodhams et al. 2008), and is consistent with other work on differences in individual susceptibility to other amphibian diseases (Pearman et al. 2004). However, this also could be an artifact of our experimental design (ending at 40 days) and is therefore worthy of further study.

Bd strains did not differ in their virulence to wood frogs in this study, in contrast to much of the published literature suggesting variability in virulence by strain (Berger et al. 2005, Retallick and Miera 2007). Because we minimized the effects of repeated passage in culture on strain virulence by using cryo-archived stock cultures, this suggests that some strains that are believed to be less virulent because of their limited effects on local populations, may be more virulent under more favorable environmental conditions for *Bd* growth.

This study exemplifies the complexities inherent when populations are coping with multiple stressors. In this

case, the perceived stressor, glyphosate-based herbicide, did not seem to suppress immune function in the host, but instead may have relieved the host from disease-caused effects by acting more strongly on the pathogen itself. This suggests caution when invoking multiple stressors as a cause for increased disease susceptibility and subsequent amphibian population declines. Our results also emphasize that the effects of multiple stressors on disease outcome depend on the relationships of individual stressors to both the pathogen and the pathogen host.

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