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Parasite-induced vulnerability to predation in larval anurans

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ABSTRACT: Within communities, pathogens and parasites have the potential to indirectly influence predator–prey interactions. For instance, prey that exhibit pathology or altered traits (e.g. behavioral shifts) following infection could be more prone to predation, which is known as parasite-induced vulnerability to predation (PIVP). PIVP has been frequently documented for pathogens with trophic transmission, because predators are often critical in the pathogen's life cycle. However, for pathogens without trophic transmission, PIVP can lead to a healthy herds effect, thereby reducing transmission in the system. In this study, we explored whether the pathogen ranavirus (family *Iridoviridae*) enhances vulnerability of 4 species of larval amphibians (spring peepers *Pseudacris crucifer*, gray treefrogs *Hyla versicolor*, American toads *Anaxyrus americanus*, and northern leopard frogs *Lithobates pipiens*) to 2 common tadpole predators (larval green darners *Anax junius* [hereinafter *Anax*] and adult water bugs *Belostoma flumineum* [hereinafter *Belostoma*]). For each anuran species, we conducted short-term microcosm experiments to assess predation rates on individuals that were or were not exposed to virus. For 3 of the 4 species, we found that exposure to ranavirus decreased survival rates with *Anax* between 2- and 9-fold. However, we did not see the same trend with *Belostoma*, which indicates that predator identity is important in this interaction. More specifically, the higher efficiency of *Anax* in capturing and consuming prey, relative to *Belostoma*, may allow *Anax* to capitalize on trait changes induced by virus exposure and enhance the PIVP effect. Our results indicate that trait-mediated indirect effects could play a role in creating healthy herds in amphibian communities.

KEY WORDS: Disease ecology · Parasite · Predation rate · *Iridoviridae* · Trait-mediated indirect effect · Parasite-induced vulnerability

1. INTRODUCTION

Predation and disease are fundamental processes in ecological communities (Holt & Dobson 2006, Morin 2011). Because predators and pathogens frequently co-occur in nature and because both may act on a single victim, these natural enemies may directly and indirectly interact to influence food web dynamics (Duffy et al. 2005, Johnson et al. 2006, Cáceres et al. 2009). In particular, disease can indirectly influence predator–prey interactions via pathology from infections. For example, sickness behaviors induced by infections could change the vulnerability of hosts to predation (reviewed in Hoverman & Searle 2016). Parasite-induced vulnerability to predation (PIVP) is often observed in systems with trophic transmission; for instance, the parasite *Toxoplasma gondii* alters the behavior of rodents, making them more susceptible to predation by cats (Berdoy et al. 2000). Because these parasites typically have complex life cycles, trophic transmission and predation serve to propagate disease. For pathogens without trophic transmission, increased vulnerability to predation can function to reduce disease risk in a population. Indeed, PIVP has the effect of both decreasing host density and reducing infection in the

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system (Hudson et al. 1992, Packer et al. 2003). Thus, predators can play a significant role in maintaining healthier herds and lowering disease risk in certain systems.

While PIVP has been reported in several systems (Hudson et al. 1992, Murray et al. 1997, Johnson et al. 2006, Duffy 2007), there have been few attempts to compare the strength of PIVP across different predator species consuming the same prey species or across different prey species that share the same predator species. Importantly, predators differ in how they encounter, capture, handle, and consume prey, which influences predation rates and overall risk posed to prey (McPeek 1990, Sih et al. 1998). Given these differences, we might expect the ability of predators to capitalize on prey vulnerabilities induced by pathogens to vary by species. Likewise, host species may vary not only in their susceptibility to pathogens (Grosholz 1992, Blaustein et al. 2005), but also in their relative vulnerability to predation (Moitoza & Phillips 1979). By assessing multiple predator species in conjunction with multiple prey species, we can determine the conditions necessary for complex interactions such as PIVP.

Amphibians provide an ideal study system for addressing the interactive effects of pathogens and predators because they are likely to encounter multiple natural enemies. Amphibian larvae encounter a diverse predator community, and a rich body of literature has documented predator–prey interactions in this system (reviewed in Relyea 2003). For instance, activity levels are correlated with predation rates; more active individuals and species are more susceptible to predation than those that are less active (Lawler 1989, Skelly 1994, Relyea 2001a). Amphibians also are threatened by several pathogens capable of causing massive mortality events (Daszak et al. 1999). In particular, viruses in the genus *Ranavirus* (family *Iridoviridae*) have been associated with epizootic events across the globe (Duffus et al. 2015). Ranavirus transmission can occur through direct contact, necrophagy, and exposure to shed virions in the environment. Ranaviruses tend to be more virulent in larval amphibians than adults, yet there is substantial variation in disease outcomes among species (Hoverman et al. 2011, Gray & Chinchar 2015). Importantly, pathology associated with ranavirus in tadpoles includes erratic swimming, lethargy, loss of equilibrium, edema, and hemorrhaging (Gray et al. 2009). Given the pathology associated with infections, there is the potential for the virus to either enhance or diminish the susceptibility of hosts to predation.

Few studies have addressed the effects of ranaviruses on species interactions and communities (Gray et al. 2009). In particular, only a single study has examined the influence of ranavirus exposure on predation rates (Parris et al. 2004). In that study, salamander larvae infected with ranavirus (Ambystoma tigrinum virus, ATV) had lower mortality rates with dragonfly larvae compared to uninfected larvae, contrary to the prediction of PIVP (Parris et al. 2004). However, there was no difference in the activity level of infected and uninfected larvae in the presence of caged dragonfly larvae. Haislip et al. (2011) also documented that virus exposure had only limited effects on behavioral responses to caged predators in 4 species of larval anurans. Because virus exposure appears to have limited effects on anti-predator behavioral responses, differences in predation rates could be related to pathology associated with infection. For instance, larval anurans exhibit lethargy as well as bursts of erratic activity when infected (Gray et al. 2009). Lethargy could lower predation rates with visually oriented predators by reducing encounter rates, which would be consistent with the findings of Parris et al. (2004). Alternatively, lethargy could impair the ability of larvae to escape predators once encountered. Moreover, bursts of erratic activity could enhance predation rates by increasing detectability. In order to deepen our understanding of these interactions and develop generalities, we must examine additional amphibian species and predators.

To assess how virus exposure influences predation rates, we conducted short-term microcosm experiments with tadpoles of 4 amphibian species and 2 predators, dragonfly larvae *Anax junius* (hereinafter *Anax*) and adult water bugs *Belostoma flumineum* (hereinafter *Belostoma*). *Anax* are considered high-risk predators because of their high capture efficiency and short handing time, which is defined as the time from prey capture to interest in the next prey item (Relyea 2001b). In contrast, *Belostoma* are low-risk predators because of their poor capture ability and long handling time. These differences in risk translate into differences in predation rates between the 2 predators, and different effects on prey traits, the magnitude of which are correlated with risk level (Relyea 2001a). We hypothesized that tadpoles exposed to virus would have higher mortality rates with predators compared to unexposed tadpoles because of the pathology associated with infection (i.e. lethargy and erratic movements). Additionally, we expected these effects to be stronger for the high-risk predator compared to the low-risk predator because of their differences in foraging efficiency.

Our focal species were spring peepers *Pseudacris crucifer*, gray treefrogs *Hyla versicolor*, American toads *Anaxyrus americanus*, and northern leopard frogs *Lithobates pipiens*. We selected these species because they commonly co-occur in wetlands, represent a range of predator-avoidance strategies, and vary in their susceptibility to ranavirus (Relyea 2001a, Hoverman et al. 2011, Wuerthner et al. 2017). Thus, this suite of species allowed us to assess generality in PIVP in this system.

2. MATERIALS AND METHODS

2.1. Species collection and maintenance

Spring peepers, gray treefrogs, American toads, and northern leopard frogs were collected from ponds surrounding the Purdue Wildlife Area (PWA), West Lafayette, IN, USA. We collected spring peepers (n = 26 pairs) and gray treefrogs (n = 28 pairs) in amplexus during breeding activity and placed each pair into a 15-l tub filled with 2 l of UV-irradiated, filtered well water to oviposit overnight in the laboratory. The pairs were released the next morning. We maintained the hatchlings in the lab until they were free-swimming, at which point they were transferred to 100-l outdoor culture pools filled with 70 l of aged well water. We collected partial American toad (n = 15) and northern leopard frog (n = 18) egg masses the morning after breeding activity and placed them into outdoor 100-l culture pools filled with 70 l aged well water. We fed tadpoles TetraMin (for early stages; Tetra) or rabbit chow (Purina) ad libitum until the experiments began.

Larval green darner dragonflies and adult water bugs were collected from ponds surrounding the PWA and housed individually in 1-l cups filled with 0.8 l of UV-irradiated, filtered well water until the experiment. We fed each predator 1 tadpole every other day. We alternated the species identity of the feeder tadpole for each feeding to ensure predators had exposure to all 4 species.

2.2. Virus culture

We used a frog virus 3 (FV3)-like ranavirus strain isolated from an infected green frog *Rana* (*Lithobates*) *clamitans* found at the PWA. Previous research has shown that this virus strain is capable of infecting each of our focal species (Pochini & Hoverman 2017, Wuerthner et al. 2017). We cultured the virus on fathead minnow cells and Eagle's minimum essential media containing 5% fetal bovine serum (MEM) to a titer of 1.68×10^6 PFU ml^{-1}. The virus was stored at −80°C until used in the experiments.

2.3. Experimental setup

Our experiment was designed to examine the effect of virus exposure on predation rates for each amphibian species. The experimental design consisted of 6 treatments: a virus- and predator-free control (Control), virus exposure only (Virus), 1 *Anax* (*Anax*), 1 *Belostoma* (*Belostoma*), virus-exposed tadpoles with 1 *Anax* (Virus + *Anax*), and virus-exposed tadpoles with 1 *Belostoma* (Virus + *Belostoma*). These 6 treatments were replicated 4 times for 24 experimental units per species (96 total experimental units). The experimental units were 15-l tubs, filled with 7 l of aged well water. The tubs were housed on racks in a covered area shaded from sunlight outside of the laboratory, spatially blocked (n = 8 replicates per block) by shelf height (n = 3 shelf heights) with experimental units randomly assigned to treatment within each block. We added 4.5 g of oak leaves (*Quercus* spp.) to each tub to provide structure and refuge. Before these leaves were added, they were soaked for 4 d to remove tannins. After soaking, we allowed 4 d for the leaves to settle to the bottom of the tubs.

Before adding tadpoles to the experimental units, we initiated the virus exposure. We began by randomly selecting 240 individuals per amphibian species and separating them into groups of 40, which we placed into 2-l tubs. These tubs were filled with 1 l of UV-irradiated, filtered well water. Four days before the experiment began, half of these tubs (n = 3 per species) were exposed to 5.95 ml of virus in Eagle's MEM (original titer: 1.68×10^6 PFU ml^{-1}) to achieve a final concentration of 10^4 PFU ml^{-1}. This dosage has been found to be sufficient for initiating infection in these species (Hoverman et al. 2010). The remaining tubs served as controls and were exposed to an equivalent volume of sterile MEM. After 2 d of exposure, the tadpoles were placed into their respective experimental units (n = 10 tadpoles per unit).

The predators were added to the appropriate tubs 1 d after the tadpoles were added. To acclimate the tadpoles to the predator's presence, the predators were initially caged within the experimental units in 1-l cups covered with mesh screen and fed 1 conspecific tadpole. Caged predators release cues that are

used by tadpoles in the formation of inducible defenses against predators (Petranka et al. 1987, Relyea 2001a). After 1 d, each predator had eaten and was released into its experimental unit. Thus, prior to the release of the predator into the experimental units, tadpoles were 4 d post exposure to ranavirus. No virus-induced mortality occurred prior to the release of the predators. Concurrently with predator release, a subsample of tadpoles (n = 10 per species) exposed only to virus was euthanized, weighed, staged, and dissected for quantitative PCR (qPCR) analysis of infection. Initial masses (means ± 1 SE) of American toads, northern leopard frogs, spring peepers, and gray treefrogs were 0.036 ± 0.005, 0.108 ± 0.011, 0.105 ± 0.014, and 0.035 ± 0.003 g, respectively. Initial average Gosner stages were 30, 25, 32, and 26, respectively.

The tubs were checked every 24 h for mortality and prey consumption. This was accomplished by visually inspecting the contents of each tub and gently lifting the tub to look on the bottom and under the leaves. The number of tadpoles seen was recorded, as were the number of dead tadpoles found in each tub. In the predator treatments, we only found 2 dead tadpoles during the experiment, and both were in *Belostoma* treatments. These tadpoles were removed from the experimental unit and preserved in 70% ethanol. Additionally, they were censored in our survival analyses.

The experiment ran for 8 d, and tadpoles were fed ad libitum throughout. At the end of the experiment, surviving tadpoles were euthanized using a 0.8 g l^{-1} concentration of MS-222 and preserved in 70% ethanol. We then staged and weighed all tadpoles, and dissected those that had been exposed to virus and a subsample from control treatments. All tadpoles exposed to virus, except for those consumed by predators, had their liver and kidneys removed and frozen at −80°C. For the sham-exposure treatments, we took a random subsample of 5 individuals per unit for ranavirus testing (n = 20 per species). For each individual, the pooled kidney and liver samples were used for virus testing. DNA was extracted from these samples using DNEasy Blood and Tissue Kits (Qiagen) and stored at −80°C until qPCR analysis.

2.4. Ranavirus testing

We used qPCR to test for ranavirus infection in the experiment (Wuerthner et al. 2017). The reaction was carried out in 96-well plates, with 4 standards, 1 negative control, and 43 experiment samples, all run in duplicate. All duplicates agreed for this experiment. Each well contained 6.25 µl SsoAdvanced Universal Probes Supermix (BioRad), 2.75 µl autoclaved Nanopure water, 1.0 µl of a mixture of each primer at 10 pmol ml^{-1} (rtMCP-F: 5′-ACA CCA CCG CCC AAA AGT AC-3′; rtMCP-R: 5′-CCG TTC ATG ATG CGG ATA ATG-3′) and a fluorescent probe (rtMCP-probe: 5′-CCT CAT CGT TCT GGC CAT CAA CCA-3′), and 2.5 µl of DNA template or autoclaved Nanopure water for a final volume of 12.5 µl. We ran qPCR reactions using a Bio-Rad real-time PCR system. The DNA standard was a synthetic double-stranded 250 bp fragment of the highly conserved *Ranavirus* major capsid protein (MCP) gene (gBlocks Gene Fragments; Integrated DNA Technologies). A standard curve was created using a log-based dilution series of 4.014×10^6 to 4.014×10^3 viral copies ml^{-1}.

2.5. Statistical analysis

We performed all statistical analysis using R version 3.4.4 (R Development Core Team 2019). To prepare the data for analysis, we combined our final survival counts with the number of tadpoles we had observed as dead and unconsumed. To best detect interaction modifications, we split the data based on victim species and by predator in a 2 × 2 factorial (e.g. spring peepers + *Anax*, spring peepers + *Belostoma*). We constructed multiplicative hazards models to examine differences in time to death for tadpoles, as a proxy for predation rates, using the packages 'survival' and 'timereg' in R (Therneau 2013, Scheike et al. 2019). We used survival over time as our response variable and included the presence of a predator, exposure to virus, and their interaction as predictors, and clustered observations by tank to account for correlation between observations within the same unit. Additionally, we calculated the expected survival for Predator + Virus treatments for each species at each time point (Billick & Case 1994, Sih et al. 1998) and compared expected survival to observed survival for the Predator + Virus treatments using Wilcoxon-Gehan tests. Expected survival was calculated by assessing additive mortality with the formula $n(p_1 + p_2 - p_1p_2)$, where n is the original number of individuals (at time 0), p_1 is the proportion of individuals experiencing mortality in treatment 1, and p_2 is the proportion experiencing mortality from treatment 2. In the case of northern leopard frogs, there was a significant difference detected between expected and observed mortality,

but not an interaction between virus and *Anax* in the multiplicative hazards model. For this case, there was low virus-treatment mortality, so we implemented a multiplicative hazards model using only Predator and Predator + Virus treatments to determine the additional hazard attributable to virus. This was done to ensure we detected any deviation from additivity, and to attach a coefficient to the detected increase in predation risk (Soluk & Collins 1988, Billick & Case 1994). Survival curves were produced using the package 'survminer' in R (Kassambara & Kosinski 2018).

3. RESULTS

3.1. Tadpole survival

For spring peepers, *Anax* increased the risk of mortality 12-fold compared to the control (Table 1, Fig. 1). Additionally, the risk of mortality with *Anax* increased 9-fold for tadpoles that we exposed to virus compared to those that were not exposed. We also found a significant difference between the expected and observed mortality in the combined *Anax* + Virus treatment ($\chi^2 = 13.7$, $p < 0.001$; Fig. 2), demonstrating that virus exposure enhanced mortality risk with *Anax*. *Belostoma*, on the other hand, did not significantly influence the risk of mortality compared to the control, nor was there an interaction between Virus and *Belostoma* (Table 1, Fig. 1). Moreover, there was no difference between expected and observed mortality for the *Belostoma* + Virus treatment ($\chi^2 = 1.00$, $p = 0.311$; Fig. 2).

For gray treefrogs, exposure to virus alone caused a significant increase in mortality over time, with mortality surpassing the control at hour 120 of the experiment (Table 1, Fig. 1). *Anax* increased the risk of mortality 22.6-fold compared to the control. Moreover, the risk of mortality with *Anax* increased 8.5-fold for tadpoles that we exposed to virus compared to those that were not exposed. Subsequent analyses found a significant difference between the expected and observed mortality in the combined *Anax* + Virus treatment ($\chi^2 = 30.6$, $p < 0.001$; Fig. 2). This demonstrates significant risk enhancement in the *Anax* + Virus treatment. For *Belostoma*, the risk of mortality increased 8-fold with the predator compared to the control. However, there was no difference in the risk of mortality with *Belostoma* for exposed and unexposed tadpoles. Interestingly, there was a marginally significant difference between expected and observed mortality in the *Belostoma* + Virus treatment ($\chi^2 = 3.9$, $p = 0.05$; Fig. 2), with observed mortality slightly lower than expected.

For leopard frogs, *Anax* increased the risk of mortality 105-fold compared to the control (Table 1, Fig. 1). However, there was not a significant increase in mortality risk in the Virus and *Anax* + Virus treatments. Despite these findings, there was a significant difference between expected and observed mortality for the *Anax* + Virus treatment ($\chi^2 = 7.6$, $p = 0.005$; Fig. 2). Because virus mortality was low in the Virus treatment (2 ind. vs. 1 ind. in the control), we compared the *Anax* and *Anax* + Virus treatments using a Cox proportional hazards model to estimate the additional hazard resulting from virus exposure. Virus exposure increased mortality risk with *Anax* by a factor of 2 ($\beta = 0.679$

Table 1. Multiplicative hazards model coefficients, robust SEs, and p-values for spring peepers *Pseudacris crucifer*, gray treefrogs *Hyla versicolor*, northern leopard frogs *Lithobates pipiens*, and American toads *Anaxyrus americanus*, compared to the control treatment. **Bold**: significant differences. Gray treefrog virus coefficients vary by time, and so are not estimated by the model. *Anax*: *A. junius*; *Belostoma*: *B. flumineum*; na: not applicable; Virus: ranavirus

Species	Factor	Coefficient	SE	p
Spring peeper				
	Anax	2.50	0.539	**<0.001**
	Virus	−1.11	1.040	0.283
	Anax × Virus	2.21	1.060	**0.037**
	Belostoma	0.786	0.790	0.319
	Virus	−1.110	1.320	0.397
	Belostoma × Virus	1.760	1.430	0.219
Gray treefrog				
	Anax	3.12	0.451	**<0.001**
	Virus	na	na	**<0.001**
	Anax × Virus	2.14	1.050	**0.041**
	Belostoma	2.070	0.489	**<0.001**
	Virus	na	na	**<0.001**
	Belostoma × Virus	0.962	0.681	0.158
Northern leopard frog				
	Anax	4.650	0.871	**<0.001**
	Virus	0.703	1.080	0.516
	Anax × Virus	−0.075	1.100	0.946
	Belostoma	3.680	0.821	**<0.001**
	Virus	0.706	0.982	0.472
	Belostoma × Virus	−0.902	1.020	0.376
American toad				
	Anax	4.840	0.985	**<0.001**
	Virus	1.850	1.080	0.087
	Anax × Virus	−1.640	1.110	0.139
	Belostoma	4.100	1.170	**<0.001**
	Virus	1.840	1.250	0.142
	Belostoma × Virus	−1.870	1.270	0.141

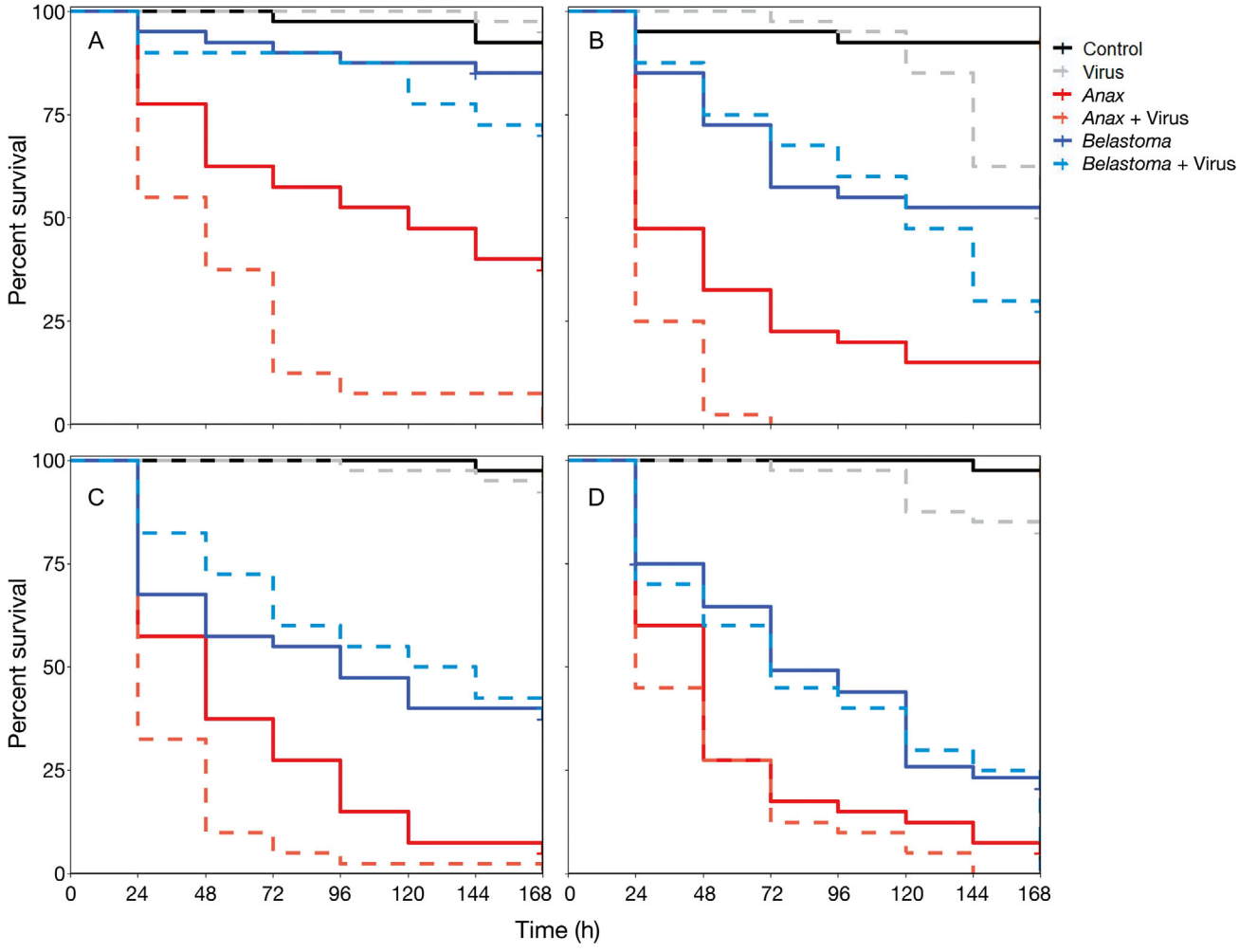

Fig. 1. Survival curves for (A) spring peepers *Pseudacris crucifer*, (B) gray treefrogs *Hyla versicolor*, (C) northern leopard frogs *Lithobates pipiens*, and (D) American toads *Anaxyrus americanus* in the 6 treatments. *Anax*: *A. junius*; *Belostoma*: *B. flumineum*; Virus: ranavirus

± 0.301, p = 0.024). With *Belostoma*, the risk of mortality increased 40-fold compared to the control (Table 1, Fig. 1). There was no difference in the risk of mortality with *Belostoma* for exposed and unexposed tadpoles. Moreover, there was no difference between expected and observed mortality in the *Belostoma* + Virus treatment (χ^2 = 1.1, p = 0.299).

For American toads, *Anax* and *Belostoma* increased the risk of mortality 126- and 60-fold, respectively, compared to the control (Table 1, Fig. 1). Virus exposure did not significantly increase the risk of mortality in the presence of either predator. Additionally, there was no difference between expected and observed mortality for the *Anax* + Virus and *Belostoma* + Virus treatments (χ^2 < 1.2, p > 0.275; Fig. 2).

3.2. Infection prevalence

We tested a random subsample of tadpoles exposed only to ranavirus from each species to determine initial infection prevalence at the start of the experiments (n = 10 per species). For American toads, northern leopard frogs, spring peepers, and gray treefrogs, we found 30, 0, 10, and 80% infection prevalence, respectively. In addition, we tested all animals from the no-predator, virus-exposure treatment for infection at the end of the experiment. We found 38, 0, 26, and 80% infection for American toads, northern leopard frogs, spring peepers, and gray treefrogs, respectively (Table 2). Similar patterns in infection prevalence across species were observed in the survivors from the *Belostoma* treatments (Table 2). However, too few tadpoles were

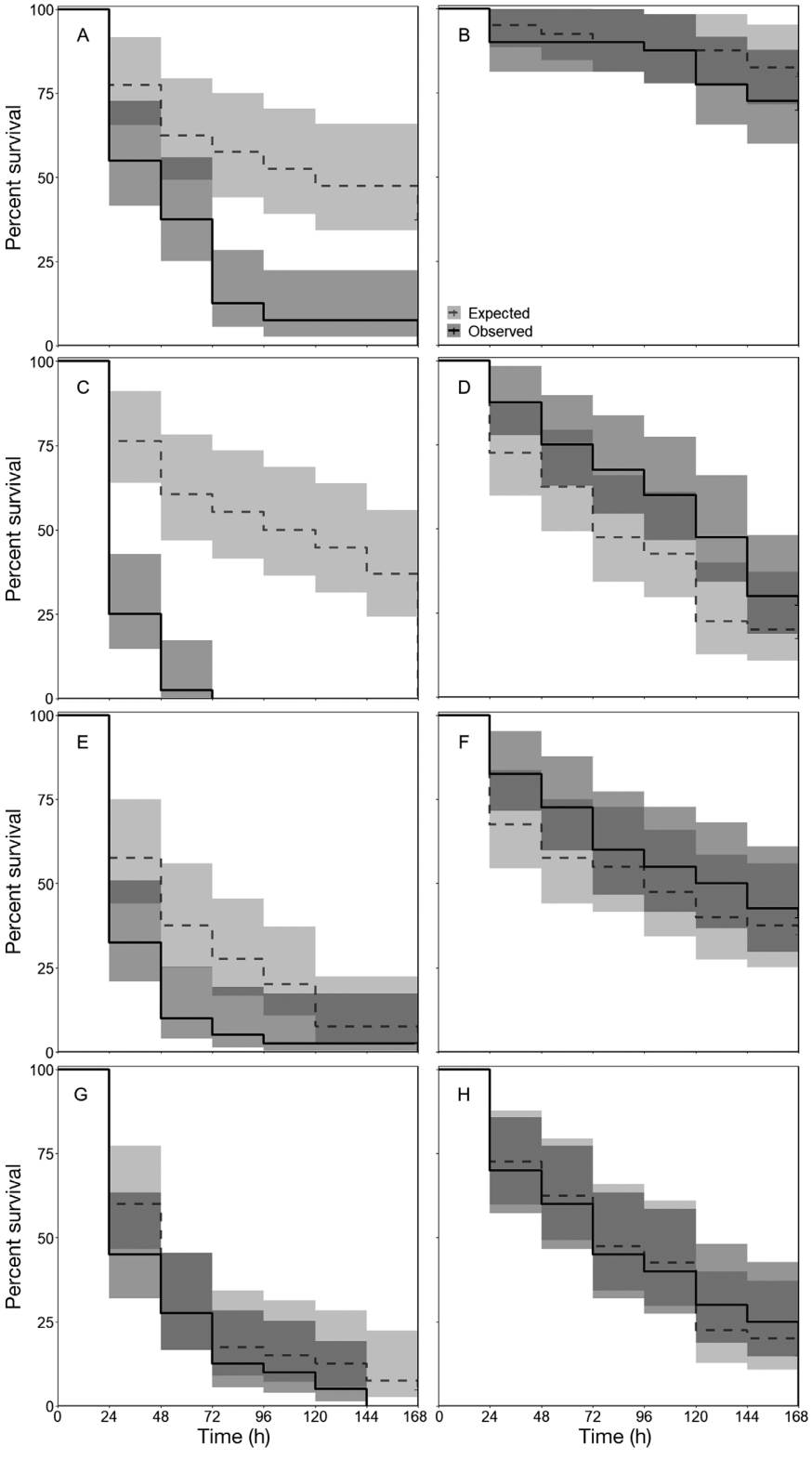

Fig. 2. Expected (dashed line) versus observed (solid line) survival curves in the Predator + Virus treatments for (A,B) spring peepers *Pseudacris crucifer*, (C,D) gray treefrogs *Hyla versicolor*, (E,F) northern leopard frogs *Lithobates pipiens*, and (G,H) American toads *Anaxyrus americanus*. Virus: ranavirus. Predator: (A,C,E,G) *Anax junius* or (B,D,F,H) *Belostoma flumineum*. Gray regions: 95% CIs

recovered alive from the *Anax* treatments to assess infection patterns. No tadpoles from no-predator, no-virus treatments tested positive for ranavirus.

4. DISCUSSION

Our results demonstrate that virus exposure can increase predation rates on larval anurans; however, the magnitude of this effect was dependent on both predator and prey identity. For 3 of the 4 amphibian species, individuals exposed to virus had between 2- and 8.5-fold higher predation rates by *Anax* compared to unexposed individuals. In contrast, there were no differences in predation rates between exposed and unexposed individuals with *Belostoma*. These differences between predators could be related to differences in their capture efficiency and handling times; *Anax* are more efficient at capturing prey and have shorter handling times compared to *Belostoma* (Relyea 2001b). Thus, *Anax* appear to be better able to take advantage of any virus-induced change in the vulnerability of tadpoles. Although we only examined 2 predator species, these results suggest that the magnitude of PIVP is dependent on the risk posed by the predator. Before generalizations can be drawn regarding types of predators that may be capable of exploiting the vulnerability induced by virus, more research should be conducted regarding both the role of predator identity and the mechanism by which this vulnerability is conveyed.

Differences in prey species identity also influenced the effect of virus on predator–prey interactions in the *Anax* treatments. Virus exposure had the largest effect on predation rates of gray treefrogs and spring peepers, moderate effects on northern leopard frogs,

Table 2. Total number of survivors (N), total number of infected survivors, and infection prevalence in the 3 predator treatments with ranavirus exposure for each prey species (spring peeper *Pseudacris crucifer*, gray treefrog *Hyla versicolor*, northern leopard frog *Lithobates pipiens*, and American toad *Anaxyrus americanus*). *Anax*: *A. junius*; *Belostoma*: *B. flumineum*; na: not applicable

Prey species	No predator			*Belostoma*			*Anax*		
	N	No. infected	Prevalence (%)	N	No. infected	Prevalence (%)	N	No. infected	Prevalence (%)
Spring peeper	39	10	26	29	12	41	3	0	0
Gray treefrog	25	20	80	12	9	75	0	na	na
Northern leopard frog	37	0	0	17	1	6	1	0	0
American toad	34	13	38	10	5	50	0	na	na

and no effect on American toads. These species-level patterns could be related to baseline activity levels of the species and how virus exposure influences behavior. For instance, spring peeper tadpoles generally have low activity levels, while American toad tadpoles are highly active (Morin 1986, Skelly 1994, Relyea 2001b). Moreover, high activity levels in prey generally influence predation rates such that highly active species will experience higher predation rates than less-active species (Lawler 1989). If virus exposure increases activity or erratic movements of prey, we would expect the effects on predation rates to be greater for prey that are generally less active. Although we did not measure behavior in our experiment to reduce disturbance and impacts on predation rates, this suggests that behavior could be mediating the observed patterns across species.

It should be noted that we only detected a single infection in northern leopard frogs at the conclusion of the experiment despite using the same virus-exposure protocol as the other species. Moreover, this same protocol was successfully used to infect northern leopard frogs in a previous study (Hoverman et al. 2011). Additionally, ranavirus infection prevalence exceeded 25% in a previous mesocosm study using this same population of northern leopard frogs (Wuerthner et al. 2017). Although infections were not detected, northern leopard frogs that were exposed to virus experienced twice the hazard of predation by *Anax*, compared to unexposed individuals. It is possible that we did successfully infect leopard frogs, but the infections were not detectable or were cleared. This would suggest that species that express resistance or tolerance to ranavirus might still experience adverse effects. Another possibility is that simple exposure to virus initiates an energetic shift towards immunity, altering prey traits as early as this initial exposure, and not requiring successful infection.

Our results are counter to the findings of Parris et al. (2004), who found that ATV reduced predation rates by *Anax* on larval tiger salamanders. The contrasting results of these 2 studies could be driven by differences in the virus or amphibian species used in the experiments. For example, ATV and FV3, 2 species of ranavirus, could influence amphibian behavior differently. It is also possible that salamanders respond differently to virus exposure than anurans. Because we did not examine behavior, we are unable to directly assess this possibility. However, Parris et al. (2004) did not detect a difference in the activity level of infected and uninfected larvae in the presence of caged *Anax*, suggesting that changes in antipredator behavior were not the driving mechanism of their predation results. To date, research examining the pathology associated with ranavirus infection has simply noted lethargy and erratic movements. Because such pathology is likely to be more subtle than standard measures of amphibian behavior (e.g. scan sampling for percent activity), there is a need to develop robust methods to quantify the effects of ranavirus on tadpole movement. For instance, video cameras and associated software can be used to track movement of individuals over the course of infection (Johansson et al. 2010, Daversa et al. 2018, Sievers et al. 2018). Given that the sensory system of predators is likely to be sensitive to fine-scale changes in prey behavior, such research will be necessary to determine the mechanisms underlying PIVP in this system.

Many species encounter multiple co-occurring natural enemies within their communities (Borer et al. 2007, Hatcher & Dunn 2011). Importantly, natural enemies can influence each other via their interactions with hosts or prey within the community (Hatcher et al. 2006). Gallagher et al. (2019) documented evidence of the healthy herds effects in amphibians such that free-ranging *Anax* reduced ranavirus prevalence in an amphibian assemblage by 83% compared to predator-free treatments. While this result appeared to be mediated by predator-

driven reductions in host density and transmission, the results of the current study demonstrate that virus exposure could also enhance the vulnerability of prey to predation. For host populations, higher consumption rates of virus-exposed individuals by predators could reduce pathogen transmission within natural systems. However, an additional question that must be addressed is whether predators selectively consume infected over uninfected prey. While selective predation is not a requirement for the healthy herds effect to occur, it does strengthen the magnitude of the effect (Packer et al. 2003). Collectively, these results suggest that a combination of density-mediated and trait-mediated effects may contribute to the healthy herds effect in amphibian communities. In light of our study, future work should focus on the role of predator density and identity, as well as understanding how community composition influences these dynamics.

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