EcoHealth
DOI: 10.1007/s10393-011-0682-1

EcoHealth

Original contribution

Combined Effects of Virus, Pesticide, and Predator Cue on the Larval Tiger Salamander (*Ambystoma tigrinum*)

Jacob L. Kerby,[1] Alison J. Hart,[2] and Andrew Storfer[2]

[1]*Biology Department, University of South Dakota, 414 E. Clark St, Vermillion, SD 57069*
[2]*School of Biological Sciences, Washington State University, Pullman, WA 99163*

Abstract: Emerging diseases and environmental contamination are two of the leading hypotheses for global amphibian declines. Yet few studies have examined the influence of contaminants on disease susceptibility, and even fewer have incorporated the role of natural stressors such as predation. We performed a factorial study investigating the interaction of the insecticide carbaryl, dragonfly predator cue, and the emerging pathogen *Ambystoma tigrinum* virus (ATV) on fitness correlates and disease susceptibility in tiger salamander larvae. Four week old larvae were exposed for 22 days in a 2 (0, 500 μg/l carbaryl) × 2 (control, predator cue water) × 2 (0, 1×10^4 pfu ATV) factorial designed laboratory study. Results show significant impacts to survival of larvae for both virus and predator cue treatments, as well as an interactive effect between the two, in which predator cue strongly exacerbated disease-driven mortality. There was a clear pattern of reduced survival with the addition of stressors, with those where all three stressors were present exhibiting the worst effects (a decrease in survival from 93 to 60%). On those that survived, we also detected several sub-lethal impacts in mass, SVL, and development. Predator cue and pesticide treatments significantly reduced both SVL and mass. Virus and predator treatments significantly slowed development. Stressors also exhibited opposing effects on activity. Predator cue caused a significant reduction in activity, whereas virus caused a significant increase in activity over time. These results highlight the importance of examining combined natural and introduced stressors to understand potential impacts on amphibian species. Such stressors may contribute to the emergence of ATV in particular regions, raising concerns about the influence of pesticides on disease emergence in general.

Keywords: Predator, Ranavirus, Pesticide, Multiple stressor, Tiger salamander

Introduction

Emerging infectious diseases are increasingly recognized for their crucial role in population and even community dynamics (Daszak et al. 2000). While some pathogens can play important roles in maintaining community diversity and ecosystem function (McCallum and Dobson 1995; Lafferty et al. 2006), others can threaten biodiversity by causing host extinction (Hudson and Greenman 1998; de Castro and Bolker 2005). These diseases are found in a wide variety of taxa and little is understood about how these diseases emerge and in particular which co-factors, if any, might be involved (Daszak et al. 2003). Understanding

Correspondence to: Jacob L. Kerby, e-mail: Jacob.Kerby@usd.edu

Published online: 27 April 2011

potential factors that facilitate disease emergence can play a critical role in managing ecosystems and potentially reduce negative effects and transmission within host species populations.

Pathogens are increasingly implicated in amphibian declines and extinctions (Daszak et al. 2000; Fisher et al. 2009). In fact, amphibians face the highest risk of extinction among all vertebrate classes (roughly 30%), and emerging infectious diseases are hypothesized as a leading cause (Stuart et al. 2004). Several other factors are implicated in these declines such as climate change, pollution, and habitat destruction. While many of these stressors are typically studied separately, there is a recent push to examine the potential for interaction between some of these factors (Forson and Storfer 2006a; Pounds et al. 2006; Lips et al. 2008; Rohr et al. 2008) and emerging disease.

One pathogen group, ranaviruses (family Iridiviridae), has been recognized for its important influence on amphibian population dynamics (Daszak et al. 2003; Stuart et al. 2004). The ranavirus, *Ambystoma tigrinum virus* (ATV), is responsible for epizootics of the Tiger Salamander, *Ambystoma tigrinum,* across much of Western North America (Jancovich et al. 2005; Storfer et al. 2007). Specifically, several ranavirus die offs have been noted to occur in agricultural areas (Green et al. 2002). Strains of the virus are thought to be spread via the bait trade, and the significance of its impact has caused it to become internationally notifiable pathogens (Jancovich et al. 2005; Storfer et al. 2007; Picco and Collins 2008). Therefore, other stressors known to exacerbate the impacts of this important pathogen are important to consider in managing the host species.

Pesticides are also implicated in amphibian population declines, but their effects are less clear, partly due to the fact that many pesticides are used in various combinations in the environment (Relyea 2009). This makes their potential role in amphibian declines somewhat enigmatic (Collins and Storfer 2003), but nonetheless, pesticides are found at detectable levels even in the least disturbed areas (Bradford et al. 2010). Given *A. tigrinum* typically inhabit ponds found surrounded by agricultural areas (Knutson et al. 2004), pesticides are potentially a significant stressor for amphibians breeding in these areas. Given the widespread occurrence of ATV across the United States, there are ample locations where pesticides and the virus will co-occur. Therefore, pesticides can potentially interact with other factors such as pathogens, either additively or synergistically. Several recent papers have shown that contaminant exposures significantly increase susceptibility of amphibian larvae to both infection and mortality from ATV exposure. Forson and Storfer (2006a, b) show that the presence of atrazine increases susceptibility to viral infection in *Ambystoma maculatum* and *A. tigrinum*. Kerby and Storfer (2009) show an additive impact on susceptibility of tiger salamanders to ATV with the addition of atrazine and chlorpyrifos. Alternatively, each of these factors might act to cancel one another out by acting antagonistically. Pesticides might impact the pathogens more strongly than the hosts, providing a protective effect when combined.

Although these direct effects are important for understanding the role of pathogens in community dynamics, indirect effects may also be important (Hudson and Greenman 1998; Lafferty et al. 2006). For example, there are several widely known examples that document pathogen influence on host behavior so as to increase disease spread (for review see Grenfell and Dobson 1995). In the tiger salamander/ATV system, Parris et al. (2004) show that infected larvae are much more effective at avoiding predation than uninfected larvae. However, it is unclear whether predators are avoiding infected prey, or if an infected prey is somehow better at avoiding predators. Depending on the system, predators can intentionally select to consume or not consume infected prey (Pfennig et al. 1998; Das et al. 2008). However, few studies have examined whether the presence of predators increases the risk of infection. In addition, there is little known even about the ecological interactions of predators and pathogens/parasites (Parris and Beaudoin 2004; Raffel et al. 2010).

There is a rich literature examining the responses of amphibians to predatory cues, summarized in a review by Kats and Dill (1998). Perhaps one of the most striking results in this regard was significantly reduced survival of tadpoles exposed to non-lethal concentrations of the insecticide carbaryl and a caged predator relative to carbaryl alone (Relyea and Mills 2001). Carbaryl is a widely used insecticide that is often found in amphibian habitats, as well as in amphibians themselves (Bradford et al. 2010). Repeated studies over several other anuran species showed that this result was common, but not ubiquitous (Relyea 2003). However, effects of carbaryl and predators have yet to be examined in conjunction with disease or in urodeles. If predatory stress can prevent larvae from surviving with a chemical contaminant that is non-lethal by itself, it might also strongly impact the ability to fight off infection. If "Two Stressors are Far Deadlier than One" (Sih et al. 2004a), then three might even be worse. Or perhaps the

incorporation of multiple stressors might prove antagonistic to one another if, for instance, the host is tolerant of pesticide exposure but the pathogen is not.

Few, if any studies have examined the impact of both a predator and a pesticide on disease susceptibility in any taxon. Studies examining subsets of two of these three factors show negative impacts, and we hypothesize that combining all three will result in even more severe effects. As anthropogenic stressors are increasingly integrated into native habitats, knowledge of their impact will become fundamental to basic understanding of population and community dynamics. In this study we ask three questions: (1) what impacts do predators have on disease susceptibility? (2) What impacts does the insecticide carbaryl have on this susceptibility? And (3) what are the effects of both pesticide and predator on the susceptibility of salamander larvae? Specifically, we examine the impacts of virus, predator cue, and pesticide exposure to tiger salamander larvae survival, growth, development, and behavior.

Methods

Salamander Larvae

Ambystoma tigrinum melanostictum egg masses were collected in the field from ponds in Teton County, WY. Embryos were reared individually in round polyethelyne containers (12.7 cm × 7.6 cm) filled with 500 ml of artesian spring water treated with Reptisafe (Zoo Med Laboratories, San Luis Obispo, CA, USA). Complete water changes were performed weekly both during the rearing period and the experiment. Larvae were maintained on a 12:12 h light:dark cycle in an environmental chamber kept at 20 ± 1°C and were fed bloodworms ad libitum three times a week. The experiment began once larvae reached Watson and Russell (2000) stage 15 (mass = 0.33 g ± 0.004), roughly 30 days following hatching. Each container held a 7.6 cm long, 1.2 cm diameter PVC pipe cut in half lengthwise to serve as a refuge for larvae.

Experimental Design

A fully factorial 2 (pesticide/no pesticide) × 2 (predator cue/no predator cue) × 2 (virus/no virus) design was used, replicating each treatment 15 times with individual animals as replicates, for a total of 120 animals. Larvae were individually housed in plastic cups filled with 500 ml treated artesian spring water. Pesticide aliquots were administered first, with virus aliquots immediately following. Using a prior protocol (Kerby and Storfer 2009), we administered to each individual cup an aliquot of either control cell media or media containing the viral strain MEL, which originates from a location near the population that our experimental salamanders were drawn from (Yellowstone National Park, WY). Larvae in the viral treatment were exposed to 1×10^4 plaque forming units of the virus via water bath for 7 days (estimated LC50; Brunner et al. 2005). We reapplied the insecticide after each weekly water change, but virus exposure occurred only during the initial week. This mode of virus exposure has resulted in significant infection in previous experiments (Forson and Storfer 2006a, b; Storfer et al. 2007; Kerby and Storfer 2009). The experiment was concluded after 22 days, when mortality had subsided in virus treatments for three consecutive days. Surviving larvae were euthanized at the end of the experiment with a water bath overdose of MS-222.

Pesticide Preparation

Pesticide applications were applied following weekly water changes to each cup individually from a newly created stock solution. We purchased Sevin (22.5% AI carbaryl) from a local hardware store and diluted it 100× with nanopure water to create a 2.25 g/l stock solution concentration. We then added 111.11 µl of stock solution to each 500 ml cup for a final concentration of 500 µg/l AI carbaryl. All stock solution concentrations were verified via gas chromatography (University of Idaho Analytical Science Laboratory, Moscow, ID, USA). This concentration represents an environmentally relevant concentration. Due to the multiple factors utilized in the design, we were limited to only a single concentration. We selected 500 µg/l as a concentration that seemed a reasonable middle range exposure. Carbaryl concentrations in aquatic habitats have been measured as high as 4.8 mg/l (Norris et al. 1983; Peterson et al. 1994) and previous studies have used similar and much higher concentrations than 500 µg/l (Relyea and Mills 2001; Relyea 2003).

Predator Cues

Dragonfly naiads (*Anax junius*) were obtained from a pond in Latah County, ID. Dragonflies were held in similar individual plastic cups as above but with 2 cm square wholes cut on two sides covered by mesh to allow flow through of water and chemicals. Twenty of these cups

containing dragonflies were submerged in two aerated 20 gallon aquaria (10 in each). Each dragonfly was fed with one *A. tigrinum* larvae per week to enhance the potential impact of the cue. No other food item was provided to insure consumption of the larvae. Thirty-six hours prior to the experiment, the dragonfly cups were drained and moved into an aerated trash can filled with 125 l of fresh water (treated as above). This time was to allow the water to obtain sufficient amounts of predator cue without fouling. Dragonflies were provided one larvae in the cup immediately prior to moving them to the trash can. ''Non-predator cue'' treatments had water held and treated similarly with the exception that no dragonfly naiads were present in the cups. Complete water changes were done in this way weekly for all treatments.

Viral Load Quantification

We monitored the condition of individuals daily, removing deceased organisms immediately and preserving them in 95% ethanol. From the tail tissue, we quantified virus loads and infection status of individuals using quantitative real-time PCR (qPCR). Methodology of DNA extraction and qPCR followed Forson and Storfer (2006a). Viral DNA was extracted via DNeasy kits (Qiagen) and quantified via a Nanodrop spectrophotometer. Samples were diluted to 20 ng/l concentrations and examined in triplicate. Reactions contained 100 ng template DNA, 300 nmol forward primer, 900 nmol reverse primer, 240 nmol probe, and Taqman 2× Universal PCR master mix (no AmpErase UNG; Applied Biosystems, Foster City, California, USA). Reactions were run for 40 cycles of 95°C denaturing (20 s), 54°C annealing (20 s), and 72°C extension (30 s) on an ABI 7300 Real-time PCR System using Real-time PCR System Sequence Detection Software version 1.2.3 (Applied Biosystems, Foster City, California, USA). All virus exposed animals were examined along with a random sampling of 20% of the no virus controls to verify lack of cross-contamination.

Variables Measured

At the beginning and end of the experiment, we measured mass, SVL, and developmental stage of each larva. On these days, we also examined the behavior of the larvae by doing spot checks each hour for four consecutive hours. Larvae were given 4 h to acclimate to new water before behavior was monitored. Larvae were scored on proportion of time in refuge, and on activity by estimating distance moved in a 5 s interval by total body lengths. A zero score was given for no movement, a score of 1 was given for up to and including 1 body length of distance moved, and so on up to a score of 5. Activity score was averaged over the 4 h and then compared among weeks.

Statistical Analyses

We used a logistic regression to detect significant interactions between treatments of virus, pesticide, and predator using the proportion dead in each treatment (PROC GENMOD, SAS 9.2). We used a 3-way MANOVA (PROC GLM, SAS 9.2) to examine impacts of these same three factors on larval mass, length, and development. Virus load quantification was compared in virus exposed treatments using a 2-way ANOVA (PROC GLM, SAS 9.2) on log transformed data examining differences in pesticide and predator treatments. The behavioral variables of activity and refuge use were analyzed via repeated measures 3-way ANOVA (SYSTAT 11) using week measured for time and comparing between pesticide, predator, and virus treatments.

Results

Mortality and Infection

There was a significant effect of virus and predator cue on larval mortality, and a near significant effect of carbaryl (Table 1). Additive effects are visible with the addition of each stressor, with a synergistic effect detected between virus and predator cue (Fig. 1, Table 1). There was a perfect correlation between individuals that died and those that were infected. That is, every infected individual died and every non-infected individual lived. Comparisons of viral load among infected (dead) individuals were similarly high and not significantly different among treatments (pesticide: $F_{1,9} = 0.47$, $P = 0.51$; predator: $F_{1,9} = 0.45$, $P = 0.52$; pesticide × predator: $F_{1,9} = 0.48$, $P = 0.51$).

Size and Development

There was a strong significant overall effect in the 3-way MANOVA for all three factors (virus: $F_{3,110} = 14.39$, $P = 0.0001$; predator: $F_{3,110} = 6.92$, $P = 0.0003$; pesticide: $F_{3,110} = 5.61$, $P = 0.0013$). Univariate outputs are provided for each variable (Table 2). Larvae in no-pesticide treat-

Table 1. Statistical results for the logistic regression of survival

Source	DF	Chi-square	P
Virus	1	15.09	*0.0001*
Predator	1	7.92	*0.0049*
Pesticide	1	3.32	0.068
Virus × predator	1	4.19	*0.04*
Virus × pesticide	1	0.63	0.43
Predator × pesticide	1	1.69	0.19
Virus × predator × pesticide	1	0.61	0.44

These results also apply to infection status since all infected individuals died. Values in italics represent significant differences.

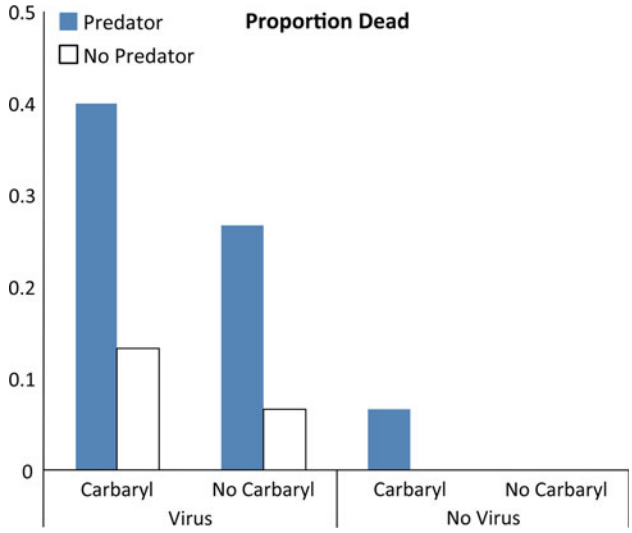

Figure 1. The proportion of salamander larvae dead in each of the treatment combinations of virus, predator and the pesticide carbaryl. A synergistic effect was detected with predator and virus treatments, and an additive negative impact of carbaryl is apparent. Each treatment contained 15 individuals.

ments were slightly larger in both mass and snout vent length than larvae in pesticide treatments (Table 3). Larvae in no-predator treatments were also slightly larger in both mass and snout vent length than those in predator treatments. However, while larvae in no-virus treatments were more developed than larvae in virus treatments they were smaller in mass. An interactive effect between virus and predator treatments was detected for snout vent length where the combination of virus and no predator cue exhibited higher growth (Fig. 2).

Behavior

Predator treatments exhibited a significantly lower average activity score (1.47 ± 0.09) than did no predator treatments (1.88 ± 0.10; $F_{1,112} = 6.44$, $P = 0.013$). Whereas virus treatments alone did not show a significant difference in activity ($F_{1,112} = 0.45$, $P = 0.50$), there was an interaction of virus x time ($F_{1,112} = 3.99$, $P = 0.049$), with activity slightly increasing in virus treatments while slightly decreasing in no virus treatments (Fig. 3). No other treatments or interactions exhibited significant interactions either by treatment or over time (Table 4). On the first day, very few of the larvae in any treatments were found in refuge. During the following week, larvae were found using refugia, but there were no significant differences among treatments (Table 2).

Discussion

This study strongly suggests that multiple stressors can alter the susceptibility of hosts to pathogens. We show important findings: (1) the presence of predator cue with virus

Table 2. Univariate outputs for length, mass, developmental stage, and refuge use

Source	Length		Mass		Development		Refuge use	
	F-ratio	*P*	F-ratio	*P*	F-ratio	*P*	F-ratio	*P*
Virus	1.87	0.17	31	*0.0001*	6.43	*0.013*	0.096	0.757
Predator	3.99	*0.048*	4.87	*0.029*	18.48	*0.0001*	0.096	0.757
Pesticide	5.26	*0.024*	16.89	*0.0001*	1.47	0.22	2.397	0.124
Virus × predator	4.93	*0.028*	0.59	0.44	0.6	0.44	0.863	0.355
Virus × pesticide	0.66	0.42	0.01	0.94	0.3	0.58	0.863	0.355
Predator × pesticide	0.07	0.80	0.16	0.69	0.01	0.91	2.397	0.124
Virus × predator × pesticide	1.00	0.32	0.22	0.64	0.11	0.74	0.096	0.757

Values in italics represent significant differences.

Table 3. Descriptive statistics for length, mass, developmental stage for each of the three examined factors of virus, predator, and pesticide

	Length		Mass		Development	
	Mean	SE	Mean	SE	Mean	SE
Virus	0.996	0.007	*0.883*	*0.013*	*15.45*	*0.137*
No virus	0.983	0.007	*0.792*	*0.012*	*15.833*	*0.083*
Predator	*0.98*	*0.007*	*0.819*	*0.013*	*15.317*	*0.122*
No predator	*0.998*	*0.006*	*0.856*	*0.014*	*15.967*	*0.092*
Pesticide	*0.979*	*0.007*	*0.804*	*0.013*	15.55	0.12
No pesticide	*1.00*	*0.006*	*0.871*	*0.013*	15.733	0.111

Values in italics represent significant differences between the two levels of a particular factor.

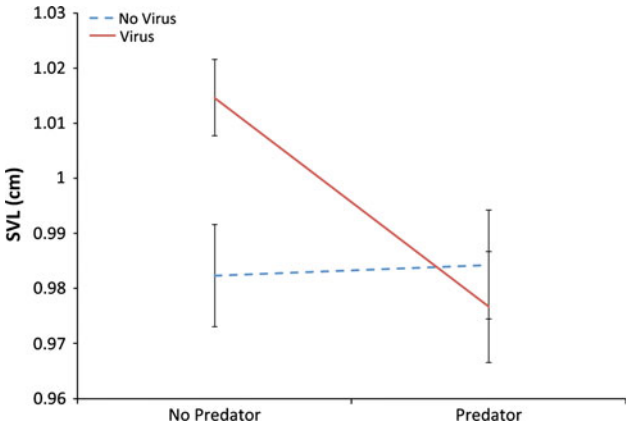

Figure 2. Differences in snout vent length of larval salamanders in virus and predator treatments. A significant interaction effect was detected with the difference in length in virus treatments disappearing with the addition of a predator cue stressor. *Error bars* represent ± 1 SE.

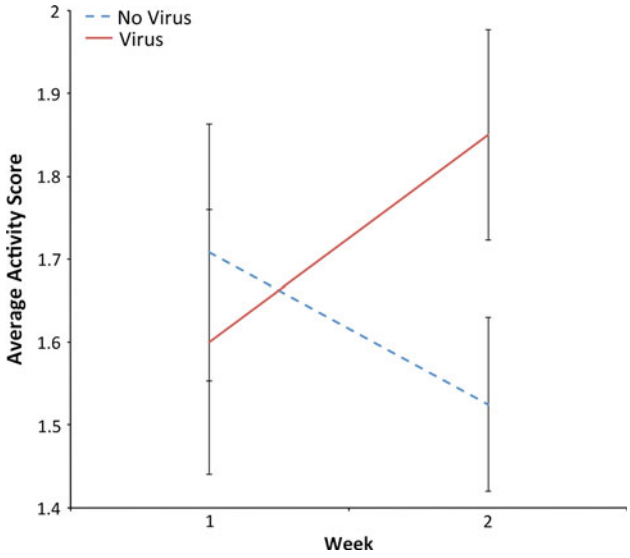

Figure 3. Differences over time in activity score of larvae exposed to virus. Virus exposed larvae are slightly more active than non-exposed larvae after 1 week of exposure. *Error bars* represent ± 1 SE.

results in a dramatic alteration in behavior, development, and survival, (2) the addition of carbaryl as a stressor has an additive negative impact on survival, and (3) the combination of multiple stressors provides a very different outcome than when each are examined individually.

Predator Cue and Virus

Relyea and Mills (2001) demonstrated that predator cues can have key implications for survival when combined with another potentially lethal factor such as a pesticide. Our study further exemplifies the lethal impacts of predator cue when combined with other stressors. It is the first to show that predator cue can dramatically increase mortality in virus exposed individuals, from approximately 7 to 27% (Fig. 1). The inclusion of the third factor of carbaryl exposure has merely an additive but nonetheless striking increase in mortality to 40%. While one might expect this increase in larval mortality to impact population dynamics (Sih et al. 2004b), disease may further increase population impacts. Theoretically, an increase in disease susceptibility can also increase transmission rates and dramatically increase overall prevalence (Fenton et al. 2002). This study did not specifically examine transmission, but understanding how these dynamics play out at a larger spatial scale with interacting individuals is an important next step.

Although it has previously been demonstrated that contaminants can alter disease susceptibility, it is of particular interest that predator cue alone can have such dramatic impacts. Relyea and Mills (2001) show that

Table 4. Results from the repeated measures 3-way ANOVA examining activity scores over a 2 week period

Source	F-ratio	*P*
Between subjects		
Virus	0.453	0.502
Predator	6.441	*0.013*
Pesticide	1.814	0.181
Virus × predator	0.097	0.757
Virus × pesticide	0.775	0.38
Predator × pesticide	0.011	0.918
Virus × predator × pesticide	0.097	0.757
Within subjects		
Week	0.094	0.76
Week × virus	3.968	*0.049*
Week × predator	0.71	0.401
Week × pesticide	0.992	0.321
Week × virus × predator	3.381	0.069
Week × virus × pesticide	0.094	0.76
Week × predator × pesticide	2.348	0.128
Week × virus × predator × pesticide	0.023	0.878

Values in italics represent significant differences.

predatory stress decreases survival in the presence of the same insecticide we used, carbaryl, although the actual mechanism is unknown. It is possible that this same type of physiological ''stress'' phenomenon is also occurring to increase susceptibility to virus in our study. An increase in the corticosterone is a typical hormonal response by tadpoles to stress (Belden et al. 2003). Belden and Kiesecker (2005) have demonstrated that glucocorticosteroid-mediated immunosuppression in tadpoles results in a higher infection of trematode parasites. If such a glucocorticosteroid response is common among amphibians, more research is necessary to understand the environmental triggers of such a response to better predict potential additive effects.

Pesticide and Virus

Whereas we did not see a significant impact of predator cue on carbaryl toxicity as is seen in the Relyea studies, we did have some mortality in this combined treatment while having no mortality in the carbaryl only treatment (Fig. 3). Nonetheless, the addition of carbaryl to any combination of other stressors further reduced survival relative to the control. This is of particular significance given that without other stressors, this specific concentration of carbaryl had no lethal impact.

As expected, carbaryl did significantly reduce mass, although unexpectedly resulted in a slight increase in length (Table 3). Snout vent length was also seen to vary significantly with both virus and predator exposure. Exposure to virus in predator-free treatments also results in a significant increase in SVL. Interestingly, this difference disappears in treatments with both predators and virus (Fig. 2). It is not entirely clear as to why an increase in length may be an adaptive response to stress, other than possibly decreasing time to metamorphosis to avoid a stressful larval environment (Werner and Anholt 1993).

Also noteworthy was the perfect correspondence of infectivity and mortality. With this particular interaction of population and viral strain, the hosts had low susceptibility to infection, but once infected, experienced 100% mortality. Since we did not examine live animals during the course of the experiment for virus infection, it is possible that some animals were infected and then subsequently lose their infection. Given the sensitivity of the qPCR test and the length of the experiment though, any infection that was cleared likely did not reach a very high load. Interestingly, the infection rate was lower than that seen in other studies (Brunner et al. 2005; Forson and Storfer 2006a; Kerby and Storfer 2009), but nonetheless, increasing evidence suggests that local host-virus dynamics vary extensively in the tiger salamander-ATV system (Storfer et al. 2007; Chojnacki, unpublished data; Ridenhour and Storfer 2008).

It is clear that the study of multiple stressors on populations and communities is increasingly needed due to the multivariate and ubiquitous nature of human environmental disturbance. The examination of simultaneous stressors is necessary in order to untangle the interaction of their combined effects. Laboratory studies such as this one allow for multiple stressors to be tested without significant cost or space. Clearly, following up this study with a larger mesocosm based study should provide a more realistic measure of population-level outcomes. Clearly in a larger arena, other effects, even antagonistic interactions between factors, might play a significant role. Nonetheless, the dramatic decrease in larval survival when exposed to multiple stressors is likely to play an important role in current amphibian declines. Given that the vast majority of amphibian species now experience both natural stressors (such as predators) and artificial stressors (such as contaminants), multiple stressor studies such as this are an important next step in understanding amphibian declines.

Clearly, amphibian species are not the only taxon that has experienced increases in both contaminant exposure

and emerging infectious diseases. Given that several avian fauna often occupy the same wetland areas as many amphibians, understanding how these same contaminants might impact their susceptibility to disease is an important aspect to consider. While there is ample evidence of direct pesticide poisoning to many bird populations (Fleischli et al. 2004), no clear link has been made to pesticide exposure and disease emergence. Clearly, there might be no link between the two, but there also seems to be no studies examining this potential link either. This also extends to other vertebrates such as fish or mammals. Increased work in a field of disease ecotoxicology might provide valuable insights to both ecotoxicologists examining the impacts of contaminants and disease ecologists attempting to find reasons for increases in emerging infectious diseases.

Acknowledgments

Thanks to the Center for Integrated Biotechnology and NSF grant 0548415 to A.S. for their sponsorship of this research. We would also like to thank K. Benyo, J. Cotter, and J. Eastman for collecting tiger salamander larvae; and J. Baumsteiger, R. Featherkile, R. Artest, A. McCally, and N. Sinacore for their logistic help.

References

Belden L, Kiesecker J (2005) Glucocorticosteroid hormone treatment of larval treefrogs increases infection by Alaria sp. *trematode cercariae*. *Journal of Parasitology* 91:686–688

Belden L, Moore I, Mason R, Wingfield J, Blaustein A (2003) Survival, the hormonal stress response and UV-B avoidance in Cascades Frog tadpoles (Rana cascadae) exposed to UV-B radiation. *Functional Ecology* 17:409–416

Bradford DF, Stanley K, McConnell LL, Tallent-Halsell NG, Nash MS, Simonich SM (2010) Spatial patterns of atmospherically deposited organic contaminants at high-elevation in the southern Sierra Nevada Mountains, California, USA. *Environmental Toxicology and Chemistry* 29:1056–1066

Brunner J, Richards K, Collins J (2005) Dose and host characteristics influence virulence of ranavirus infections. *Oecologia* 144:399–406

Collins J, Storfer A (2003) Global amphibian declines: sorting the hypotheses. *Diversity & Distributions* 9:89–98

Das K, Roy S, Chattopadhyay J (2008) Effect of disease-selective predation on prey infected by contact and external sources. *BioSystems* 95:188–199

Daszak P, Cunningham A, Hyatt A (2000) Emerging infectious diseases of wildlife-threats to biodiversity and human health. *Science* 287:443

Daszak P, Cunningham A, Hyatt A (2003) Infectious disease and amphibian population declines. *Diversity & Distributions* 9:141–150

de Castro F, Bolker B (2005) Parasite establishment and host extinction in model communities. *Oikos* 111:501–513

Fenton A, Fairbairn J, Norman R, Hudson P (2002) Parasite transmission: reconciling theory and reality. *Journal of Animal Ecology* 71:893–905

Fisher M, Garner T, Walker S (2009) Global emergence of Batrachochytrium dendrobatidis and amphibian chytridiomycosis in space, time, and host. *Annual Review of Microbiology* 63:291–310

Fleischli M, Franson J, Thomas N, Finley D, Riley W (2004) Avian mortality events in the United States caused by anticholinesterase pesticides: a retrospective summary of National Wildlife Health Center records from 1980 to 2000. *Archives of environmental contamination and toxicology* 46:542–550

Forson D, Storfer A (2006) Atrazine increases ranavirus susceptibility in the tiger salamander, *Ambystoma tigrinum*. *Ecological Applications* 16:2325–2332

Forson D, Storfer A (2006) Effects of atrazine and iridovirus infection on survival and life-history traits of the long-toed salamander (*Ambystoma macrodactylum*). *Environmental Toxicology and Chemistry* 25:168–173

Green D, Converse K, Schrader A (2002) Epizootiology of sixty four amphibian morbidity and mortality events in the USA, 1996 2001. *Annals of the New York Academy of Sciences* 969:323–339

Grenfell B, Dobson A (1995) *Ecology of infectious diseases in natural populations*, Cambridge: Cambridge Univ Press

Hudson P, Greenman J (1998) Competition mediated by parasites: biological and theoretical progress. *Trends in Ecology & Evolution* 13:387

Jancovich J, Davidson E, Parameswaran N, Mao J, Chinchar V, Collins J, et al. (2005) Evidence for emergence of an amphibian iridoviral disease because of human-enhanced spread. *Molecular Ecology* 14:213–224

Kats L, Dill L (1998) The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience* 5:361–394

Kerby J, Storfer A (2009) Combined effects of atrazine and Chlorpyrifos on susceptibility of the Tiger Salamander to Ambystoma tigrinum virus. *EcoHealth* 6:91–98

Knutson M, Richardson W, Reineke D, Gray B, Parmelee J, Weick S (2004) Agricultural ponds support amphibian populations. *Ecological Applications* 14:669–684

Lafferty K, Dobson A, Kuris A (2006) Parasites dominate food web links. *Proceedings of the National Academy of Sciences* 103:11211

Lips K, Diffendorfer J, Mendelson J III, Sears M (2008) Riding the wave: reconciling the roles of disease and climate change in amphibian declines. *PLoS Biology* 6:e72

McCallum H, Dobson A (1995) Detecting disease and parasite threats to endangered species and ecosystems. *Trends in Ecology & Evolution* 10:190–194

Norris L, Lorz H, Gregory S (1983) Influence of forest and range land management on anadromous fish habitat in western North America: forest chemicals. General technical report PNW, Portland, OR

Parris M, Beaudoin J (2004) Chytridiomycosis impacts predator-prey interactions in larval amphibian communities. *Oecologia* 140:626–632

Parris M, Davis A, Collins J (2004) Single-host pathogen effects on mortality and behavioral responses to predators in salamanders (Urodela: Ambystomatidae). *Canadian Journal of Zoology* 82:1477–1483

Peterson H, Boutin C, Martin P, Freemark K, Ruecker N, Moody M (1994) Aquatic phyto-toxicity of 23 pesticides applied at expected environmental concentrations. *Aquatic Toxicology* 28:275–292

Pfennig D, Ho S, Hoffman E (1998) Pathogen transmission as a selective force against cannibalism. *Animal Behaviour* 55:1255–1261

Picco A, Collins J (2008) Amphibian commerce as a likely source of pathogen pollution. *Conservation Biology* 22:1582

Pounds J, Bustamante M, Coloma L, Consuegra J, Fogden M, Foster P, et al. (2006) Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* 439:161–167

Raffel T, Hoverman J, Halstead N, Michel P, Rohr J (2010) Parasitism in a community context: trait-mediated interactions with competition and predation. *Ecology* 91:1900–1907

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

Relyea R (2009) A cocktail of contaminants: how mixtures of pesticides at low concentrations affect aquatic communities. *Oecologia* 159:363–376

Relyea R, Mills N (2001) Predator-induced stress makes the pesticide carbaryl more deadly to gray treefrog tadpoles (Hyla versicolor). *Proceedings of the National Academy of Sciences* 98:2491

Ridenhour B, Storfer A (2008) Geographically variable selection in Ambystoma tigrinum virus (Iridoviridae) throughout the western USA. *Journal of evolutionary biology* 21:1151–1159

Rohr J, Raffel T, Romansic J, McCallum H, Hudson P (2008) Evaluating the links between climate, disease spread, and amphibian declines. *Proceedings of the National Academy of Sciences* 105:17436

Sih A, Bell A, Kerby J (2004) Two stressors are far deadlier than one. *Trends in Ecology & Evolution* 19:274–276

Sih A, Kerby J, Bell A, Relyea R (2004) Response to Schmidt. Pesticides, mortality and population growth rate. *Trends in Ecology & Evolution* 19:460–461

Storfer A, Alfaro M, Ridenhour B, Jancovich J, Mech S, Parris M, et al. (2007) Phylogenetic concordance analysis shows an emerging pathogen is novel and endemic. *Ecology Letters* 10:1075–1083

Stuart S, Chanson J, Cox N, Young B, Rodrigues A, Fischman D, et al. (2004) Status and trends of amphibian declines and extinctions worldwide. *Science* 306:1783–1786

Watson S, Russell A (2000) A posthatching developmental staging table for the long-toed salamander, *Ambystoma macrodactylum krausei*. *Amphibia-Reptilia* 21:143–154

Werner E, Anholt B (1993) Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. *American Naturalist* 142:242