Single-host pathogen effects on mortality and behavioral responses to predators in salamanders (Urodela: Ambystomatidae)

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Abstract: Pathogens can alter host behavior and affect the outcome of predator–prey interactions. Acute phase responses of hosts (e.g., a change in activity level or behavioral fever) often signal an infection, but the ecological consequences of host behavioral changes largely are unexplored, particularly for directly transmitted (i.e., single-host) pathogens. We performed three experiments to test the hypothesis that a pathogen, Ambystoma tigrinum virus (ATV), alters host behavior of Sonoran tiger salamanders (Ambystoma tigrinum stebbinsi Lowe, 1954) and enhances predation. In the first experiment, salamander larvae exposed to ATV experienced 48% lower mortality from dragonfly Anax junius (Drury, 1773) larvae than those in controls. Second, uninfected and infected larvae exposed to the nonlethal (caged) presence of predators did not significantly differ in their distance from the predator. Infected salamanders significantly increased their activity level relative to those in controls in predator-free conditions. Finally, ATV-infected larvae preferred significantly warmer temperatures than uninfected larvae, but larvae reared at the thermal maximum for the virus all died. High host activity level yet retention of effective antipredator responses likely benefits ATV because this single-host pathogen relies on host survival for transmission. Preference for warmer temperatures may be associated with the host response to pathogens and may help fight infection.

Résumé : Les pathogènes peuvent altérer le comportement de leurs hôtes et affecter le résultat des interactions prédateurs–proies. Les réactions de phase aiguë (par exemple, un changement de niveau d’activité ou une fièvre comportementale) de l’hôte indiquent souvent l’existence d’une infection; mais, les conséquences écologiques des changements de comportement de l’hôte restent en grande partie inexplorées, particulièrement dans le cas des pathogènes transmis directement, c’est-à-dire ceux qui n’ont qu’un seul hôte. Nous avons fait trois expériences pour vérifier l’hypothèse selon laquelle le pathogène, le virus d’Ambystoma tigrinum (ATV), change le comportement de l’hôte, la salamandre tigrée du Sonora (Ambystoma tigrinum stebbinsi Lowe, 1954), et augmente la prédation. Dans la première expérience, les larves exposées expérimentalement au ATV subissent une mortalité due aux larves de libellules Anax junius (Drury, 1773) inférieure de 48 % à celles des larves témoins. Dans la seconde expérience, des larves infectées et non infectées de salamandres exposées à la présence non létale de prédateurs (en cages) ne diffèrent pas par la distance qu’elles maintiennent entre elles et les prédateurs. En l’absence de prédateurs, les salamandres infectées augmentent significativement leur niveau d’activité par comparaison aux témoins. Enfin, les larves infectées par ATV préfèrent des températures significativement plus élevées que les larves non infectées, mais celles qui ont été gardées à la température maximale du virus sont toutes mortes. Le niveau accru d’activité de l’hôte, en même temps que la rétention de réactions efficaces contre les prédateurs, sont vraisemblablement bénéfiques à ATV, parce que ce pathogène n’a qu’un seul hôte et qu’il dépend de la survie de cet hôte pour assurer sa transmission. La préférence pour les températures plus élevées peut être reliée à la réaction de l’hôte aux pathogènes et peut aider à combattre l’infection.

Introduction

Detecting and avoiding predators increases prey survival, so many taxa respond to predators by reducing activity to avoid them (Lima and Dill 1990; Skelly and Werner 1990; Skelly 1994). This adaptive response may be compromised by pathogens that alter host behavior (Thompson 1990; Lefcort and Eiger 1993; Lefcort and Blaustein 1995; Thiemann and Wassersug 2000) in ways that diminish a prey’s ability to avoid or escape predators (Giles 1983). For example, many fish and amphibians use refugia more often under the risk of predation (reviewed in Lima and Dill...
1990), but pathogens may elevate activity levels, increasing a host’s conspicuousness and risk of predation (Dolinsky et al. 1985; Quinn et al. 1987).

Ectotherms also behaviorally thermoregulate (Kluger 1991). A typical acute-phase response to pathogens is for infected hosts to prefer warmer microhabitats (i.e., behavioral fever). For example, bullfrog (Rana catesbeiana Shaw, 1802) tadpoles infected with Aeromonas bacteria choose warmer temperatures (Lefcort and Eiger 1993). Because warm aquatic conditions usually are in shallow regions with high predator densities (Arnold and Wassersug 1978), predation risk may be enhanced for feverish individuals. Thus, stereotypical responses of infected ectotherms may facilitate interspecific pathogen transmission by increasing the risk of predation for infected individuals. However, increased predation of infected individuals likely would only benefit multiple-host pathogens in which the definitive host consumes the intermediate host to complete the transmission cycle (Giles 1983; Hechtel et al. 1993). Increased interspecific predation presumably would be disadvantageous for single-host pathogens whose transmission depends on host survival. Although altered behavior may function to increase transmission rates of some single-host pathogens (e.g., rabies; Baer 1973), increasing the probability of host death ultimately would decrease fitness of the pathogen (Dobson 1988). An alternative explanation for febrile responses is that they may protect the host against pathogens. High body temperatures are deleterious to pathogens whose thermal maxima fall within a temperature range lower than the maximum a host can endure (Kluger et al. 1975).

Amphibians are ideal systems for investigating the behavioral and antipredator response to pathogens because they can be manipulated experimentally, and have recurring viral-induced epidemics in several taxa (Cunningham et al. 1996; Laurance et al. 1996; Jancovich et al. 1997; Bollinger et al. 1999). Viral epidemics are frequent in tiger salamander, Ambystoma tigrinum (Green, 1825), populations in the western United States and Canada (Jancovich et al. 1997; Bollinger et al. 1999). The single-host viral pathogen (Ambystoma tigrinum virus; ATV hereafter) is spread from sick to susceptible animals through water or by direct contact (Jancovich et al. 2001). ATV also can be transmitted in the larval stage through cannibalism, although cannibalism and disease frequency are inversely correlated in tiger salamander populations (Collins et al. 2003); thus cannibalism is unlikely a major mechanism of pathogen transmission. Our study tested the impact of ATV on antipredator behavior in larval Sonoran tiger salamanders (Ambystoma tigrinum stebbinsi Lowe, 1954). We used three laboratory experiments to test whether infection by ATV alters adaptive host antipredatory and thermoregulatory behavior.

Materials and methods

Experiment 1: virus effects on susceptibility to predation

Ambystoma tigrinum stebbinsi larvae were obtained from a laboratory breeding in June 2000. We used one clutch to minimize the number of federally protected animals necessary for our experiment. Ambystoma tigrinum stebbinsi also show minimal genetic variation (Jones et al. 1988; Storfer et al. 2004). Thus, our use of one clutch is both ethically justified and is representative of variation in natural populations, which are often highly inbred (Storfer et al. 2004). After hatching, all larvae were held before testing in plastic containers (114 L) at an approximate density of 0.11 larvae/L, fed brine shrimp (genus Artemia Leach, 1819) ad libidum, and reared on a 12 h light : 12 h dark cycle. All experimental animals were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care.

Our design tested for differences in susceptibility of uninfected and ATV-infected larval tiger salamanders to predation by dragonfly Anax junius (Drury, 1773) larvae. We administered viral infections by exposing salamander larvae for 1 week to water baths containing infectious concentrations of ATV (10<sup>3</sup> plaque-forming units; Jancovich et al. 1997). We exposed 150 larvae (30 groups of 5 larvae) to 150 mL of ATV-inoculated water in small cups. Our design therefore simulated transmission by water, a likely mode of ATV transmission in native A. tigrinum populations (Jancovich et al. 2001). An additional 30 groups of 5 larvae reared in 150-mL cups without ATV served as controls.

Treatments consisted of uninfected or ATV-infected A. t. stebbinsi reared with A. junius larvae in 15 L of water (37-L aquaria). The two treatments were replicated 13–14 times in 27 aquaria. All salamander larvae reached Harrison’s (1969) stage 46 before testing and were 10–12 mm long. Our response variable was predation; thus, disappearance or injury to salamander larvae was our measure of predation. We collected A.junius larvae with dip nets from nearby ponds (Maricopa County; 33°N, 112°W) and held them in the laboratory for 4 days to ensure adequate hunger level and predatory behavior. We reared salamander larvae in both treatments at an initial density of 10 larvae/aquarium (0.67 larvae/L), which is high but within the range of natural densities of larval tiger salamanders in Arizona (Collins and Cheek 1983; Pfennig et al. 1994). After salamander larvae acclimated for 24 h, we measured and randomly added a single A. junius larva to each predator aquarium (A. junius body length = 15.1 ± 2.4 mm; mean ± SD). We observed animals three times daily, and used the proportion of salamander larvae remaining at the end of 6 days as our estimate of predation risk. We chose a 6-day experimental period because by day 6 at least one salamander larva was eaten in all aquaria and, in some aquaria, all larvae were eaten.

Preliminary analyses indicated that A. junius body length did not significantly differ between uninfected and virus-infected salamander larva treatments (t<sub>25,0.05</sub> = 0.15, P = 0.8799). Thus, differences in predator body size likely did not affect feeding success of dragonfly larvae in our experiment (Caldwell et al. 1980). Survival data were analyzed using G statistics for log-likelihood-ratio goodness-of-fit tests on untransformed data (PROC FREQ; SAS Institute Inc. 1990) because of significant departures from normality and heteroscedasticity of error variances between uninfected and ATV-infected treatments (H<sub>G</sub>; σ<sup>2</sup>* = σ<sup>2</sup>*, F<sub>[13,12]</sub> = 4.16, P = 0.0188).

Experiment 2: virus effects on behavioral responses to predators

We used 10 uninfected and 10 ATV-infected A. t. stebbinsi larvae (see experiment 1 for larval source and infection pro-
Experiment 3: thermal preferences

We quantified the thermal preferences of uninfected and ATV-infected salamander larvae in two aluminum, trough-shaped apparatus (1.5 m x 12 cm x 10 cm) filled with approximately 8 L of water to a depth of 5 cm. The low water level prevented a vertical stratification of water temperatures. We created the warm region of a thermal gradient by placing a heat lamp underneath one end, and the cold region by packing dry ice around the other end of each trough. Heat and cold sources were centered in a 15-cm region at each end of the troughs.

We obtained 10 uninfected and 10 ATV-infected salamander larvae as in experiments 1 and 2. We randomly selected and introduced one larva into the middle of each trough, and left them undisturbed for 20 min. After the acclimation period, we noted the location of each larva and recorded the water temperatures at those positions every 2 min for 2 h. We tested each larva three times. Larvae had a 24-h rest period between trials, and were fed brine shrimp. All trials were conducted between 21 and 25 June 2000.

We used differences between responses in treatments containing empty cages and those with caged predators to test the behavior of salamander larvae in the nonlethal presence of predators. Preliminary repeated measures analyses indicated no significant effect of trial date on larval location (uninfected: $F_{[2,30]} = 1.60, P = 0.2188$; infected: $F_{[2,36]} = 1.22, P = 0.3062$) or activity level (uninfected: $F_{[2,32]} = 0.07, P = 0.9359$; infected: $F_{[2,36]} = 1.00, P = 0.3783$). We therefore considered mean measurements over the three trials as the unit of observation for all subsequent analyses of variance (ANOVA). We used multivariate analysis of variance (MANOVA) to test for the effect of the independent factor disease (ATV absent or present) on the dependent variables larval location and activity level. We then used Bonferroni-adjusted (significance level of 0.025 for two response variables) ANOVA contrasts on each response variable to determine significant contributors to the multivariate effect (PROC GLM; SAS Institute Inc. 1990).

Results

Experiments 1 and 2

Dragonfly larvae consumed significantly more uninfected than infected salamander larvae ($G_1 = 20.17, P < 0.0001, N = 270$; Fig. 1). MANOVA indicated a significant effect of infection status on combined larval responses; however, only significant differences between uninfected and infected larvae in activity level, but not location, contributed to the multivariate effect (Table 1). Within treatments, neither uninfected ($F_{[1,15]} = 0.31, P = 0.5847$) nor ATV-infected ($F_{[1,18]} = 1.79, P = 0.1977$) larvae differed significantly in their proximity to a cage when it had predators versus when it was empty (Fig. 2A). The significant univariate infection status effect on activity level was driven mainly by differences between predator treatments for ATV-infected larvae. Activity levels did not significantly differ for uninfected larvae across predator treatments, but infected larvae had sig-
Table 1. MANOVA and univariate ANOVA results of larval location and activity level for uninfected and ATV-infected *Ambystoma tigrinum stebbinsi* larvae exposed to either absence or presence of nonlethal predatory *Anax junius* cues.

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Note: Significance levels for univariate tests were interpreted at 0.025, using a Bonferroni correction for three response variables.

Fig. 1. Proportion of uninfected and ATV-infected *Ambystoma tigrinum stebbinsi* larvae surviving to day 6 in the presence of predacious *Anax junius* larvae. Salamander larvae were reared in groups of 10 larvae per aquarium and values plotted are least-square means ± 1 SE.

Fig. 2. (A) Average distance away from cage and (B) proportion of time spent active during 20-min trials when cages contained *A. junius* larvae and when they were empty. Values plotted are least-square means ± 1 SE for three successive trials for uninfected and ATV-infected *A. t. stebbinsi* larvae. Asterisks indicate significant differences ($P < 0.05$) between treatment means.

Experiment 3

ATV-infected larvae preferred significantly warmer temperatures than uninfected larvae ($F_{1,17} = 12.61, P = 0.0025$; Fig. 3). Uninfected and infected salamander larvae challenged with a warm environment suffered 100% mortality by day 6, while larvae reared at moderate and cold temperatures had no mortality. Within the warm environment treatment, infected larvae experienced faster mortality than uninfected larvae ($\chi^2 = 4.11, P = 0.0427$; hazard ratio = 3.453). All surviving infected larvae in behavioral and thermal preference trials tested positive for ATV; uninfected larvae had no virus.

Discussion

Our experiments demonstrate that behavioral responses to infection likely do not increase predation risk for larval salamanders. Salamanders infected with ATV exhibited stereotypical acute-phase responses to infection by seeking significantly higher activity levels when exposed to an empty cage relative to a cage with *A. junius* (Fig. 2B).
warmer temperatures and increasing activity levels, but they also reduced activity under the threat of predation. These responses are expected for a single-host pathogen that does not benefit from death of its host.

Low mortality with dragonfly predators, coupled with reduced activity when exposed to predator cues, suggests that infection by ATV does not compromise the ability of salamander larvae to detect and respond adaptively to predators. Inactivity in predator environments may benefit hosts because immobility reduces predation risk from visually foraging predators (e.g., Lawler 1989), but uninfected larvae did not reduce their activity levels around predators, and infected larvae only reduced their activity level to that of uninfected larvae. This response may reflect a trade-off between avoiding predators and obtaining resources (Godin and Sproul 1988; Nicieza 2000). Low activity levels reduce foraging efficiency (Skelly 1994; Anholt et al. 1996), decrease competitive ability (Morin 1983), and prolong development (Skelly 1994), ultimately increasing the time exposed to predators. Remaining active therefore may affect host fitness in ways that override the benefits of reduced activity in the presence of visually oriented predators. However, mortality was 48% higher for uninfected larvae compared with infected animals, and differences in activity level cannot account for all of the difference. Although not tested in our study, differential mortality may be a result of predators avoiding infected individuals.

The lower predation rate of infected larvae likely is adaptive for viral transmission. Jancovich et al. (2001) demonstrated that ATV is a single-host pathogen that is transmitted between conspecifics without interspecific predation. If the normal host defense response to predation benefits the pathogen by keeping the host alive, then we expect even infected hosts to retain their predator-avoidance behaviors. Multiple-host pathogens typically show increased fitness by changing the host’s behavior to make it more vulnerable to predators (Dobson 1988; Lafferty 1992). The benefits of enhancing host mortality for a one-host pathogen such as ATV are likely negligible because only salamanders are a host for the virus (Jancovich et al. 2001; Brunner et al. 2004). Infected salamander larvae increased activity by 58% in predator-free environments. Single-host pathogens that elevate activity levels in their host gain a selective advantage by increasing contact rates among conspecifics (Baer 1973). Thus, ATV may facilitate viral transmission through increasing host activity in the absence of predators.

Infected salamanders preferred warmer water compared with uninfected larvae. Altered thermoregulatory behavior (i.e., behavioral fever) occurs in most ectothermic vertebrates when infected with pathogens, and generally is considered an adaptive host response rather than a mechanism to enhance pathogen success (Kluger et al. 1975; Kluger 1991). Febrile responses are adaptive because they slow replication or kill pathogens (Kluger et al. 1975), although some pathogens may benefit from altered host temperature preferences (Porat et al. 1991). Jancovich et al. (1997) demonstrated that ATV grows optimally in its salamander host at 18 °C, and infectivity decreases as temperatures rise to a thermal maximum of 26 °C. Given that infected larvae selected warmer microhabitats in our experiment, behavioral fever may benefit the host by decreasing viral growth rates. Animals did not select the warm temperature extreme that would have killed the pathogen (26 °C) undoubtedly because, regardless of infection status, all animals perished when reared at the high temperature. Moreover, ATV-infected animals suffered faster mortality than uninfected larvae in the extreme warm temperature treatment, indicating a reduced ability for infected animals to cope with thermal stress. Thermal preferences therefore reflect a trade-off between minimizing pathogen proliferation while maintaining homeostasis.

Although not addressed in our experiment, increased thermal preferences likely would cause infected larvae to move towards warmer, shallow water. Lefcort and Blaustein (1995) demonstrated that larval red-legged frogs (Rana aurora Baird and Girard, 1852) infected with a single-host yeast pathogen (Candida) selected both warmer and shallower aquatic microhabitats. This response presumably is maladaptive for both host and single-host pathogens because amphibian predators often congregate in shallow water areas (e.g., Arnold and Wassersug 1978; Heinen and Hammond 1997). However, the benefits of behavioral fever may override selection for predator avoidance. Our experiments also demonstrated that ATV-infected larvae suffer lower mortality in predator environments, and therefore may take advantage of warmer temperatures while efficiently avoiding predation in shallow aquatic environments.

Behavioral and thermoregulatory responses to a single-host virus are a compromise between conflicting selection pressures on host and pathogen (Ewald 1983). Altered thermoregulatory behavior could be adaptive for the host as a measure for eliminating an infection (Kluger et al. 1975). Elevated activity levels may increase pathogen transmission by increasing contact rates among healthy and infected individuals. Single-host pathogens may benefit from increased activity without predators and also benefit from reduced host activity with predators.
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References


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