PREVALENCE OF *AEROMONAS HYDROPHILA* IN FROGS AND TOADS AT THE PIERCE CEDAR CREEK INSTITUTE

FINAL URGE REPORT TO THE PIERCE CEDAR CREEK INSTITUTE

PREPARED BY:

DEAN MCCURDY, PH.D. AND KRISTEN KRUM

BIOLOGY DEPARTMENT, ALBION COLLEGE

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Introduction

Amphibians are in decline globally and the causes of the declines are poorly understood (reviewed by Green et al., 2002). In the Great Lakes region, the disease 'red-leg' has been implicated in mass-mortality of captive and natural populations of frogs (Forbes et al., 2004). This disease is typically associated with the presence of the bacterium *Aeromonas hydrophila*, which is ubiquitous in aquatic environments, but only leads to mass mortality in some instances (e.g., Bradford, 1991). One possibility is that *Aeromonas* is an opportunistic generalist parasite which takes advantage of amphibians with weakened immune systems (possibly the result of stresses associated with environmental change; Rollins-Smith, 2001). Links between environmental change, stress, and parasitism have recently been suggested as explanations for sudden epizootics of malaria in birds and epizootics of trematodes in populations of invertebrates (e.g., McCurdy et al., 1998; McCurdy, 2001). Researchers have also suggested that opportunistic parasites can have sudden, drastic impacts to host populations and even alter the structure of ecological communities (e.g., McCurdy and Moran, 2004). Thus, it is important to document the presence of potentially pathogenic organisms, even when epizootics are not occurring.

We proposed to determine the extent of colonization by *Aeromonas hydrophila* on adult frogs and toads at the PCCI using a low-impact, non-destructive sampling technique (catching and swabbing the skin of frogs). If colonization by *A. hydrophila* is associated with stress to frogs, we predicted that prevalence would be highest at times when frogs were breeding or when temperatures were coldest (these conditions represent known stresses for frogs; Cooper et al., 1992). We also expected that male frogs would be more likely to harbor *A. hydrophila* bacteria than females because of immunosuppression associated with testosterone in breeding males (Schalk and Forbes, 1997).

Methods

We studied prevalence of *Aeromonas hydrophila* in adult wood frogs (*Rana sylvatica*), green frogs (*Rana clamitans*), leopard frogs (*Rana pipiens*), bull frogs (*Rana catesbeiana*), pickerel frogs (*Rana palustris*), northern spring peepers (*Pseudacris crucifer*), and Fowler's toads (*Bufo fowleri*) at the PCCI between 11 April and 15 July, 2005. We located frogs by searching transects parallel to ponds and seasonally-flooded areas (cf. Forbes et al., 2004) and from opportunistic encounters. When captured, each frog was measured (snout-vent length), weighed, assessed for body condition (e.g., noting lesions, missing appendages, etc.), sexed, and rubbed on the abdomen with a sterile swab. The swab was stored in an individual container with phosphate buffer for transport to the lab (Taylor et al., 1999). The frog was then marked with an internally-visible, fluorescent elastomer dye to prevent accidental re-sampling (Nauwelaerts et al., 2000). The locations of captured frogs were noted for entry into a GIS database for the PCCI property.

In the aquatic lab at the PCCI, swabs from frogs were inoculated onto Ryan's *Aeromonas* medium at 27°C for 48-hours (Ryan's medium includes an antibiotic which kills most bacteria other than *Aeromonas* strains; Forbes et al., 2004). Bacteria that grow on the medium were then identified using a metabolic identification kit. Data presented herein are for prevalence of *A. hydrophila* (defined as the percentage of infected frogs). Data were analyzed using X^2 tests-of-independence to assess importance of factors such as sex of frogs on prevalence of bacteria. Logistic regression analysis was used to assess the likelihood of infection as a response to environmental factors (date, air temperature, water temperature).

Results

Over the summer of 2005, we captured 185 different frogs of seven different species within the PCCI property. Overall, *Aeromonas hydrophila* bacteria were isolated from the skin of 86.0% of frogs captured. Prevalence of this bacterium varied significantly across species ($X^2 = 18.1$, $df = 6$, $P < 0.01$; Table 1) and ranged from a low of 50% ($n = 16$) in spring peepers, and a high of 100% ($n = 6$) in leopard frogs. There was no overall sex-bias in prevalence of bacteria on frogs (prevalence on females = 82.5%, $n = 40$; prevalence on males = 73.2%, $n = 39$; $X^2 = 1.1$, $df = 1$, $P = 0.28$). When breaking this down by species, there was also no sex-difference in prevalence within green frogs ($X^2 = 0.03$, $df = 1$, $P = 0.86$; small sample sizes for other species precluded statistical analysis due to difficulties with sexing other species accurately). Prevalence of bacteria was unrelated to the length of frogs and frog mass (for all logistic comparisons, $P > 0.05$; similar comparisons made for each species separately also were not significant).

Mean date of capture differed across the seven species of frogs observed in our study ($F = 3.98$, $df = 6,208$, $P < 0.001$). As a result, comparisons of prevalence in relation to temperature and calendar date must be made cautiously because such relationships could be confounded by the species of frogs present at various times. For example, frogs were less likely to harbor *A. hydrophila* bacteria as the season progressed (Logistic $X^2 = 24.3$, $df = 1$, $P < 0.0001$); however, when broken down by species, this relationship was not significant for green frogs or wood frogs (the two species observed frequently at various times during the summer). Similarly, overall prevalence of bacteria increased with increasing air temperature (Logistic $X^2 = 4.2$, $df = 1$, $P < 0.05$), but not for any species when considered separately (all $P > 0.05$). Across all species, prevalence of *A. hydrophila* bacteria was not related to water temperature (Logistic $X^2 = 1.3$, $df = 1$, $P = 0.25$).

Discussion

Unexpectedly, prevalence of *A. hydrophila* bacteria on epidermal samples of frogs was very high (86%). We expected that prevalence would be similar to - or lower than prevalence recorded by researchers working at other sites because of the undisturbed nature of ponds on the PCCI property. Specifically, other researchers studying ranid frogs at sites in eastern Ontario frogs found that prevalence of *A. hydrophila* on epidermal samples of frogs ranged from 4% on green frogs to 17% on leopard frogs (Forbes et al., 2004). Hird et al. (1981) reported prevalence of 32% from juvenile and adult wood frogs in a Minnesota population. One possibility is that the method of detection we used (swabbing samples onto whole petri dishes to look for colonies of bacteria) resulted in a more sensitive test than methods used by other researchers (e.g., innoculation onto small agar slants; Forbes et al., 2004). In direct comparisons of our method with those used others (results not shown), it did appear that our method was more likely to detect infections, but this difference was not sufficient to explain the large difference in prevalence between studies. Monitoring of frog populations throughout the year, repeated sampling across years, and samples of frogs from surrounding watersheds would all help determine whether high prevalence of bacteria we observed is characteristic of frogs only on the PCCI property or applies over a larger geographical area.

Differences in prevalence of *A. hydrophila* across species are difficult to explain with our limited dataset, although we speculate that our sampling design may, in part, explain our results. For example, spring peepers were least likely to be infected, but we observed that most peepers were breeding before intensive sampling began in May. Thus, if high prevalence is positively associated with breeding (as found by Forbes et al. [2004]), we might have caught most of our spring peepers when they were under relatively lower than other frogs. Similarly, we caught

most of our leopard frogs later in the season, which coincided with their breeding season and might explain why prevalence was so high in this species. Our observations are in partial agreement with those of Forbes et al., (2004), who also observed that prevalence of *A. hydrophila* was highest in leopard frogs, and explained this as relating to their breeding season. Although we observed no obvious behavioral differences in frogs that ultimately tested positive versus negative for *A. hydrophila*, the possibility that infected frogs were more likely to be captured cannot be ruled out. We did observe several dead green frogs during our study, and those that were tested were positive for *A. hydrophila.*

We observed no difference in prevalence of *A. hydrophila* bacteria between male and female frogs, despite evidence of sex-biased parasitism across many vertebrate taxa (Schalk and Forbes, 1997; McCurdy et al., 1998). Such differences might be expected if one sex is exposed more frequently to bacteria, or from immunosuppression associated with sex hormones (mostly testosterone) (McCurdy et al., 1998). In their study, Forbes et al. (2004) also observed no difference in prevalence of *Aeromonas hydrophila* between male and female frogs. Nonetheless, it would still be valuable to investigate interactions between sex hormones, breeding stress, and parasitism, as researchers have suggested that frogs may be especially susceptible to parasites when under stress associated with breeding (this might also explain high prevalence overall, see above).

As mentioned above, we observed that prevalence of *A. hydrophila* bacteria in any given species was not related to air temperature or water temperature. This was somewhat unexpected given evidence that frogs are under stress when under colder temperatures (Cooper et al., 1992; Zapata et al., 1992) and are expected to harbor bacterial infections at that time. One explanation for this is that frogs in our study were simply overwhelmed by exposure to high levels of

bacteria, rendering seasonal differences in prevalence at our site undetectable. In one recaptured frog (a green frog caught 5 times), we did observe that infection status changed over the breeding season.

Given the high frequency of colonization of *Aeromonas hydrophila* on frogs, further research on links between *Aeromonas* infection and immunobiology of frogs is required, especially for strains that have been associated with mortality in frog populations (e.g., Huys et al., 2003). Further, there is increasing evidence that declines in amphibian populations (including in the Great Lakes area) may be related to pathogens, such as *A. hydrophila* (Forbes et al., 2004) and that *Aeromonas* infections may also render frogs more susceptible to viruses or environmental disturbances (Cunningham et al., 1996). The diversity of frog species, abundance of field sites, and high rate of prevalence of *A. hydrophila* within the PCCI property make it an ideal study site for experimental or observational studies of amphibian pathogens. In the future, it would be useful to assess measures of infection intensity in addition to prevalence (efforts to do this in our lab are underway). Experimental studies on effects of climate change on stress and prevalence of *A. hydrophila* in frogs would be especially helpful in determining the pathology of red-leg disease and its possible impacts on frog populations.

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Table 1. Prevalence of *Aeromonas hydrophila* on frogs at the Pierce Cedar Creek Institute.

Species	% positive	*n*
Bullfrog	75.0	12
Green frog	90.7	129
Leopard frog	100.0	6
Spring Peeper	50.0	16
Pickerel frog	100.0	3
Fowler's toad	84.2	19
Wood frog	86.7	30