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2005 Hixon Fellow Report

Urbanization and the Impact of Emerging Disease on Amphibians

Problem Statement

Emerging disease is a critical challenge for environmental scientists (Natl Acad Sci 2003). Accelerating urbanization of human populations is causing unprecedented rates of landscape alteration, which result in habitat fragmentation and the potential release of numerous pollutants into remaining wildlife habitat. The resulting disease emergence in both humans and wildlife is associated with these anthropogenic changes in the environment, however the mechanisms underlying these patterns remain undetermined in most cases (Dobson and Foufopoulos 2001). It is possible that the combination of pollutants and poor habitat quality may decrease host immunity and alter host and vector distribution, thereby leading to disease emergence (Dobson and Foufopoulos 2001, McKinney 2002). **The objective of my research is to examine the impact of urbanization on patterns of disease in wildlife and to investigate the underlying mechanisms.**

Amphibian declines are of growing global concern, and emerging disease and habitat loss are two of the likely causes (Blaustein and Wake 1995, Alford and Richards 1999, Houlahan et al. 2000, Stuart et al. 2004). A recent study suggests that one-third of all amphibian species are globally threatened, making the group more endangered than either birds or mammals (Stuart et al. 2004). I propose to estimate urbanization effects on patterns and consequences of infection by echinostomes, trematode parasites, on *Rana clamitans* (green frog) in Northeastern Connecticut. Echinostomes are among the most common macroparasite encountered in North American amphibians (Schmidt and Fried 1997, McAlpine and Burt 1998). Echinostomes encyst in the kidneys of many amphibian

species where it can disrupt kidney function when present in sufficient numbers. We have recently demonstrated that echinostome infection is more intense in wetlands in an urban as compared to a non-urban environment, suggesting that echinostomiasis may be an emerging disease in urbanized environments (Skelly et al. in press). The goal of my studies is to determine how echinostomes impact amphibians in nature, and to identify factors that lead to disease emergence in urban environments.

Background

Urbanization and Disease

Anthropogenic alteration of the landscape is often invoked in considering emerging disease and has been identified as a critical area of study for increasing our understanding of disease emergence (Daszak et al. 2001). Recently, urbanization and its impact on disease has been the focus of an increasing number of studies (Schrag and Wiener 1995, Gratz 1999, Daszak et al. 2001, Dobson and Foufopoulos 2001). It has been suggested that emerging disease occurrence has risen in human populations in recent years, and anthropogenic influences on the environment have been identified as a potential cause. Similar patterns of disease have also been observed in wildlife populations (Daszak et al 2000, Epstein et al. 2003, Johnson et al. 2003).

Macroparasites and Urbanization

Existing evidence suggests that macroparasite infection can increase in urban areas, but few studies have been conducted. In a recent review, we identified 27 studies that estimated the impact of urbanization on macroparasite infection; of these 14 found

increases with urbanization, 10 found declines, and 3 showed no effect (Skelly et al. in press). Of seven trematode species studied thus far, five were more prevalent in hosts in urbanized areas (Richards et al.1995, Amorim et al. 1997, Azazy and Al-Tiar 1999, Bustnes and Galaktionov 1999, Mngomezulu et al. 2002, Johnson and Chase 2004). The two trematode species that were more prevalent in rural areas were both of the genus *Schistosoma* and were examined in humans (Amorim et al. 1997, Azazy and Al-Tiar 1999, Mngomezulu et al. 2002). Therefore, the studies conducted on the impact of trematodes on wildlife populations along an urbanization gradient all demonstrate that trematode infection increases with urbanization. However, the number of studies conducted is small, and the mechanisms for increased macroparasite infection in wildlife in urban environments remain unclear.

Echinostomes and Amphibian Performance

Echinostomes decrease amphibian performance in laboratory studies, however the impact of echinostomes in nature has not been examined. Echinostomes have been reported in the kidneys of many North American anuran species (McAlpine and Burt, 1988, Beasley et al., 2003). Echinostome cercariae penetrate a broad range of second intermediate hosts, including tadpoles, and subsequently migrate to the host's kidneys, where they encyst as metacercariae (Beaver 1937, Anderson and Fried 1987, Huffman and Fried 1990, Martin and Conn 1990, Fried et al. 1997). Several laboratory studies have indicated that echinostomes slow growth and cause mortality in infected tadpoles (Fried et al. 1997, Schotthoefer et al. 2003). Schotthoefer et al. (2003) demonstrated that the impact of echinostome infection on leopard frog tadpoles is dependent on

developmental stage. In another study, echinostome infection resulted in a significant decrease in the mean length and weight of the infected tadpoles (Fried et al. 1997). Together, these studies indicate that echinostomes have a stage-dependent impact on growth and survival of leopard frog tadpoles in a laboratory setting. However, the impact of echinostomes on tadpoles in nature remains unknown.

Echinostomes and Urbanization

In a recent observational study, we determined that echinostome infection is higher in amphibians in an urban environment as compared to a rural environment (Skelly et al. in press). We examined parasites present in green frog metamorphs in 16 wetlands along an urbanization gradient in Northeastern Connecticut (Skelly et al. in press). Wetlands were located in one of three areas: Yale Myers Forest, Tolland, and Manchester. Yale Myers Forest does not contain any full-time human inhabitants, Tolland is a suburban area, and Manchester is a city. We dissected the kidneys of up to ten green frog metamorphs from each wetland and counted metacercarial echinostome cysts. Vouchers of the cysts were sent to Dr. Vasyl Tkach at the University of North Dakota for identification via molecular techniques (Tkach and Pawlowski 1999). Of the 131 green frogs examined, 38% were infected with echinostomes, and infection intensities ranged

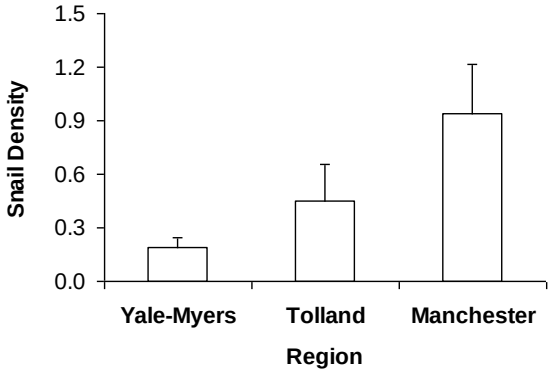

Figure 1. Variation in the density host snails in 59 Connecticut wetlands found in undeveloped, suburban and urban regions. (From Skelly et al. in press).

from 1-1648 cysts (Skelly et al. in press). The echinostome cysts occupied nearly one third of the volume of the kidneys in the most infected animals (Skelly et al. in press). Our study provides evidence for emergence of echinostomes in urbanized contexts given that greater than 99% of the dissected metacercarial cysts came from suburban or urban wetlands in Manchester or Tolland. Wetlands where echinostome infection was detected all contained snail taxa that act as intermediate hosts for echinostomes (Skelly et al. in press). We determined that host snails were five times more abundant in Manchester as compared to Yale Myers Forest, and infection was a strong function of host snail density (Linear regression $F_{1,14} = 53.638$, $P < 0.001$) (Figure 1, Skelly et al. in press). Echinostome infection abundance increased exponentially with snail density suggesting that snail density alone may not explain the observed increase in infection in urban ponds.

Snail Vector Responses

The few studies that examine the response of snail vectors to urbanization suggest that snail density can increase in urbanized environments, thereby contributing to disease emergence (Gratz 1999). Echinostome cercariae are shed from snail hosts during the spring and summer, and this free swimming stage enters tadpoles and encysts in the kidneys. Our study demonstrates that echinostome infection abundance

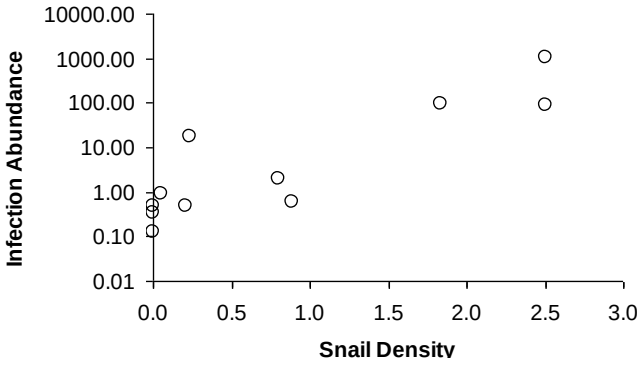

Figure 2. Relationship between the density of host snails and echinostome infection abundance within kidneys of *R. clamitans*. Infection abundance is plotted on a logarithmic scale (From Skelly et al. in press).

increases exponentially with increasing host snail density (Figure 2). This suggests that snail density is likely not the only factor responsible for the observed increase in infection in the urbanized environment. Another study shows that the trematode *Ribeiroia ondatrae*, a parasite demonstrated to cause limb deformities in many amphibian species, is more prevalent in eutrophic ponds (Johnson and Chase 2004). Studies conducted to date suggest that the impact of infection on a host decreases as a result of eutrophication (Lafferty and Holt 2003). Johnson and Chase (2004) hypothesize that eutrophication may decrease the impact of *R. ondatrae* on its snail host, thereby increasing the period of cercarial shedding. The lengthened cercarial shedding period could result in an increase in the infection intensity in amphibians (Johnson and Chase 2004).

Compromised Host Condition

In addition to changes in disease vectors, compromised host immunity can result in an increase in macroparasite infection and may be involved in amphibian decline. It is possible that pollutants have played a major role in amphibian decline given that the cause remains unknown for nearly half of amphibian species undergoing decline (Stuart et al. 2004). Several studies suggest that exposure to pollutants leads to compromised immunity in tadpoles and increased susceptibility to macroparasite infection (Christin et al. 2003). A study in which juvenile leopard frogs were exposed to a mixture of six pesticides and subsequently challenged with a parasitic nematode revealed an effect of pesticide exposure on the frogs' immune response (Christin et al. 2003). Frogs exposed to the pesticide mixture prior to being challenged with nematodes had a higher prevalence of infection than unexposed frogs (Christin et al. 2003). Another study

demonstrated that exposure of toads to malathion resulted in increased susceptibility to bacterial infection (Taylor et al. 1999). Behavioral responses to macroparasites may also contribute to a degraded immune response. Thiemann and Wassersug (2000) demonstrated that tadpoles' behavioral response to exposure to macroparasites may increase exposure to pollutants. The presence of echinostome cercariae caused tadpoles to reduce activity, which results in sinking. Sinking to the pond bottom could cause in increased exposure to chemical pollutants in the pond substrate that might weaken immune response (Thiemann and Wassersug 2000).

Approach

The studies that I conducted during my 2005 Hixon fellowship represent a portion of my doctoral dissertation. I conducted several laboratory experiments to investigate whether echinostomes affect the survival, growth or development rate of green frog tadpoles. I determined that green frog tadpoles are affected differently by echinostomes based on their developmental stage at the time of echinostome exposure. In addition, echinostome infection can result in mortality. Histopathological analysis of kidney tissue from echinostome infected tadpoles revealed potential mechanisms by which echinostomes disrupt kidney function. Results from these studies will soon be submitted to a peer-reviewed scientific journal for publication.

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