SEPARC
Information Sheet #3

THE BACTERIUM *AEROMONAS HYDROPHILA* IN AMPHIBIAN POPULATIONS

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Red Leg Syndrome in Amphibians:

Recently, biologists have been increasingly concerned with the rapid declines in amphibian populations across the globe. While habitat destruction has been an insidious factor in many declines, a multitude of other factors including climate change, introduced species, agricultural pesticides, and diseases have been cited as potential causes. Diseases in particular have been a major research focus of amphibian biologists due to the frequent observations of mass mortality events involving thousands of individuals. Indeed, amphibians are susceptible to a variety of different pathogens including fungi, viruses, and bacteria (1). Over a century ago, the red leg syndrome was identified in captive amphibian populations (2). As the name implies, amphibians that succumb to the red leg syndrome have reddening on the skin of the hindlimbs and ventrum. At the time, the suspected cause of red leg syndrome was the bacterial species *Aeromonas hydrophila*. Many bacterial species including *A. hydrophila* are common in most aquatic habitats inhabited by amphibians. However, recent research into other pathogens such as the fungus *Batrachochytrium dendrobatidis* and viruses belonging to the genus *Ranavirus* has shown that amphibians often exhibit signs of disease from these agents similar to red leg syndrome (1). Given that several types of pathogens can cause similar gross signs (e.g., red legs), there has been increased focus on determining the etiologic agent of amphibian mortality events. Recently, *A. hydrophila* was ruled out as the causative agent in 64 mortality and morbidity events in the United States from 1996 to 2001 (3). Indeed, few mortality events in free-ranging amphibian populations can be attributed to *A. hydrophila* (4,5). Rather, *A. hydrophila* appears to be an opportunistic or secondary invader that capitalizes on dead or morbid amphibians that were infected with other pathogens (1). While *A. hydrophila* does not appear to be playing a significant role in amphibian population declines, ranaculture facilities have frequently reported outbreaks of the pathogen. These outbreaks are typically attributed to improper husbandry practices that have compromised immune function and increased susceptibility to bacterial infections (4).

Pathogen Characteristics:

The bacterial species *A. hydrophila* is a Gram-negative polar flagellated rod (6). This facultative anaerobe is ubiquitous in many aquatic systems including lentic, lotic, and brackish waters that range widely in temperature, pH, salinity, and conductivity (7). *A. hydrophila* has been isolated

from healthy amphibians as well as other cold-blooded species including reptiles and fish (2,8-10).

Amphibian Immune Defenses:

Amphibians are exposed to a variety of pathogens due to their strong association with aquatic environments. However, amphibian skin can act as a potential barrier to pathogens. Amphibian skin contains antimicrobial peptides that can inhibit the growth of many microorganisms including bacteria (11). Interestingly, studies have shown that the growth of *A. hydrophila* is not inhibited by the antimicrobial peptides produced by several species of frogs (11,12). Consequently, it is hypothesized that bacteria passing through amphibian skin are targeted by other elements of the immune system (12). For example, the major histocompatibilty complex (MHC) plays an important role in the immune system of many vertebrates including amphibians. Recently, Barribeau et al. (13) demonstrated that the MHC of African clawed frog tadpoles (*Xenopus laevis*) helps confer resistance and tolerance to *A. hydrophila*. Although this research suggests that the amphibian immune system can target *A. hydrophila*, additional research examining the immune responses of North American amphibians is necessary to determine the mechanisms by which amphibians clear *A. hydrophila* infections.

Signs of Disease and Diagnostic Testing:

While *A. hydrophila* appears to be part of the natural microbiota of healthy frogs, it can become pathogenic and cause disease (4,10). Disease caused by *A. hydrophila* was historically referred to as red leg syndrome or red leg disease, but bacterial septicemia and bacterial dermato-septicemia are more appropriate terms for the disease (4). It should be noted that these latter terms are used to describe generalized bacterial infections caused by a diversity of species including *Acinetobacter* spp, *Aeromonas* spp, *Citrobacter* spp, *Flavobacterium* spp, and *Psuedomonas* spp (4). Signs of disease associated with *A. hydrophila* depend on the severity of infection (1,4). Minor infections can occur at the site of abrasions and wounds and lead to skin discoloration and ulceration (4). Systemic infection with *A. hydrophila* can lead to erythema (reddening) of the skin on the legs and ventrum. Individuals also can show edema (swelling) in the body and legs, anorexia, epidermal ulcers, and skin sloughing (5). Internal organs such as the spleen, liver, and kidneys may become enlarged or hemorrhage. Importantly, many of these signs of disease are similar to infections caused by other pathogens. Thus, it is critical that diagnostic tests (e.g., bacterial culturing, histological examination, PCR) are preformed to identify the etiological agent in any observed mortality events. Moreover, given the opportunistic nature of *A. hydrophila*, it important that specimens are collected from the field within hours of mortality and processed before colonization by decompositional bacterial species (1).

Factors Contributing to Emergence:

Healthy individuals appear to be relatively resistant to *A. hydrophila* infections, but natural and anthropogenic stressors may compromise the amphibian immune system and increase susceptibility to the pathogen. The breeding season of amphibians is a stressful period that can be associated with an increased likelihood of *A. hydrophila* infection. For example, Forbes et al.

(14) documented high prevalence of *A. hydrophila* in breeding northern leopard frogs (*Rana pipiens*). The authors suspected that aggressive encounters between competing males increased wound frequencies and sites for *A. hydrophila* infection. Forbes et al. (14) also found a trend for greater *A. hydrophila* prevalence in adult northern leopard frogs, American bullfrogs (*R. catesbeiana*), and green frogs (*R. clamitans*) that recently emerged from hibernation compared to individuals captured later in the season. Given that cold temperatures often suppress immune function in amphibians (15), many pathogens including *A. hydrophila* may capitalize on this brief period of susceptibility to infect new hosts (16). Anthropogenic factors such as pesticides also may increase susceptibility to infection. Taylor et al. (17) found that Woodhouse's toads (*Bufo woodhousi*) exposed to the insecticide malathion and injected with *A. hydrophila* experienced 70% greater mortality compared to individuals not exposed to malathion but injected with the pathogen. Moreover, stressors associated with captive husbandry of amphibians such as poor water quality, overcrowding, and transport are frequently cited in *A. hydrophila* outbreaks (1,4). Thus, despite limited evidence that *A. hydrophila* is a contributing factor in the recent decline of amphibian populations, natural and anthropogenic stressors that compromise immune function could increase the likelihood that this ubiquitous bacterial species becomes pathogenic.

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