SEPARC
Information Sheet #1

Southeastern PARTNERS IN AMPHIBIAN AND REPTILE CONSERVATION

AMPHIBIAN RANAVIRUSES IN THE SOUTHEASTERN UNITED STATES

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Amphibian Die-offs:

While amphibians are susceptible to many pathogens, viruses belonging to the genus *Ranavirus* are responsible for the majority of amphibian die-offs in North America (1). Ranaviruses cause disease in frogs, toads, and salamanders, and mass mortality events have occurred in all regions of the United States (2). Each year it is estimated that 1 – 3 new states report amphibian die-offs associated with ranaviruses (3). Moreover, the number of reported die-offs in the United States from ranaviruses is 3 – 4X greater than the fungal pathogen *Batrachochytrium dendrobatidis* (1). Thus, ranaviruses likely represent the greatest pathogen threat to the biodiversity of amphibians in North America.

In the Southeast, known die-offs from ranaviruses have occurred in the wild in Tennessee, North Carolina and Florida (4). A variety of species were affected in these mortality events including wood frogs, pickerel frogs, green frogs, American bullfrogs, green tree frogs, spotted salamanders, and marbled salamanders. Undoubtedly, additional die-offs have occurred but gone unnoticed. It is also important to note that die-offs have been reported in ranaculture facilities in Georgia and Idaho and several zoos throughout the United States (5,6,7).

The importance of ranaviruses as a significant pathogen is often dismissed because many of the reported die-offs have been with common species (1). While the frequent occurrence of reported die-offs with common species is likely a consequence of detection probability, die-offs have been reported in several species of concern in the western United States (e.g., mountain yellow-legged frog, California red-legged frog, western toad, Sonoran tiger salamander; 3,8). It is very likely that die-offs of locally rare species have occurred in the Southeast without detection. For example, it has been suspected that ranaviruses may play a role in the population dynamics of the Mississippi gopher frog, which is an endangered species.

Pathogen Characteristics:

Ranaviruses belong to the viral family Iridoviridae (9). There are three known species of *Ranavirus* that infect amphibians: *Frog virus 3*, *Bohle iridovirus*, and *Ambstoma tigrinum virus* (10). Ranaviruses are most pathogenic to amphibian larvae, although die-offs have been reported in adults in the Southeast and elsewhere (3,11). Mortality can be catastrophic, with over 95% of a population dying (3). The pathogen can be transmitted through direct contact, ingesting infected tissue, and exposure to contaminated water or sediment (12). The environmental persistence of ranaviruses is unknown but the pathogen likely can survive outside the amphibian host for over a month (13).

Signs of Disease and Diagnostic Testing:

Amphibians infected with ranaviruses often have swollen appendages or mouth parts and reddening of the skin (Fig. 1). The body cavity also may be swollen. It is important to note that these signs also can be associated with other amphibian pathogens such as bacteria (e.g., *Aeromonas hydrophila*) or alveolates. Behaviorally, amphibians infected with ranaviruses stop eating, become lethargic, and lack coordination (3). Although this pathogen can infect multiple organs, ranaviruses often target the liver and kidney. At necropsy, these organs may appear pale or mottled, and hemorrhages can be seen in the kidney (3). Ultimately, the amphibian dies due to chronic cell death in the target organs. While mortality can occur after just a few days of exposure to the virus, some individuals can be sublethally infected and function as reservoirs (14).

The only way to confirm that an amphibian is infected with ranaviruses is by conducting diagnostic tests, which may include histological examination of tissues for pathological changes, electron microscopic examination for viral particles, and use of the polymerase chain reaction (PCR) for molecular testing. These techniques are available at most diagnostic labs, and minimum cost is usually around $30 per animal (in 2009 USD) for PCR testing.

Factors Contributing to Emergence:

The first catastrophic die-offs from *Ranavirus* were reported in the mid-1990s in the southwestern United States and the United Kingdom (8,15). Since then, die-offs have been reported in over 20 U.S. states, 3 Canadian provinces, and on 4 continents other than North America suggesting that the virus is widespread and potentially emerging (2,16,17). There are two hypotheses for the emergence of ranaviruses: (1) reduced amphibian immunity associated with increased occurrence of anthropogenic stressors, and (2) introduction of novel *Ranavirus* strains into naïve amphibian populations by humans. Anthropogenic stressors could include agricultural land use, heavy metal pollution, global warming, or acid rain. Studies have found that amphibians exposed to pesticides or nitrogenous waste deposited by cattle are more likely to become infected with ranaviruses (18,19). Amphibian larvae are particularly susceptible because of their immature immune systems and strong association with wetlands, ponds and streams where these stressors can become concentrated. The introduction of novel strains of the virus into amphibian populations is a particular conservation concern (20). Humans can inadvertently transport virus particles among watersheds on footwear, clothing, or recreation equipment. Moreover, recent studies have shown that *Ranavirus* strains found in captive amphibian facilities (e.g., bait shops, ranaculture ponds, biological supply companies) are more virulent than wild strains (7,21). Because people frequently buy amphibians from captive facilities and accidentally or intentionally release them into the environment, novel and highly virulent strains can be released into naïve amphibian populations.

Conservation Strategies:

Logical conservation strategies include reducing stressors at amphibian breeding sites and decreasing the likelihood of transporting and introducing ranaviruses into naïve populations. We recommend that at least 100-ft vegetative buffers be established between agricultural operations and amphibian breeding sites. If cattle access in water systems is necessary, we recommend that their densities be maintained below 15 head per acre of surface water. To reduce the chance that novel *Ranavirus* strains are transported among amphibian populations, the soles of footwear and recreational equipment should be disinfected. Effective disinfectants are 3% bleach and 1% Novalsan® with at least 1 minute of contact time (22). Potassium permanganate is not effective at inactivating ranaviruses (22). Lastly, release of amphibians into an environment from where they do not originate should not be done. In an effort to reduce the likelihood that commercially-raised amphibians potentially infected with ranaviruses are not transported among states or countries, the World Organization for Animal Health (http://www.oie.int/) is organizing standard protocol for *Ranavirus* testing. Recently, this organization listed ranaviruses as a notifiable disease. This listing will eventually result in regulations that require a *Ranavirus*-free declaration for commercial transport of amphibians.

Figure 1. Swollen and red legs of an American bullfrog (left) and Cope's gray treefrog (right) tadpole infected with *Ranavirus*. The body cavity and mouth parts of the Cope's gray treefrog also are swollen.

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Recommended Citation:

Gray, M. J., J. T. Hoverman, and D. L. Miller. 2009. Amphibian ranaviruses in the southeastern United States. Southeastern Partners in Amphibian and Reptile Conservation, Disease, Pathogens and Parasites Task Team, Information Sheet #1.