SEPARC
Information Sheet # 17

SOUTHEAST PARTNERS IN AMPHIBIAN AND REPTILE CONSERVATION

RANAVIRUSES IN SQUAMATES

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Introduction:

Ranaviruses (family Iridoviridae, genus Ranavirus) are double-stranded DNA viruses that replicate in temperatures of 12-32 °C and may survive outside of a host in aquatic environments for months and at temperatures > 40 °C (Daszak et al. 1999, Chinchar 2002; La Fauce et al 2012, Nazir et al. 2012). They infect and can lead to mass mortality events in reptiles, amphibians and fishes (Chinchar 2002; Jancovich et al. 2005). Studies on ranavirus pathogenesis and disease ecology have focused largely on amphibians and fishes, and have demonstrated that susceptibility and severity of infection vary with age and species of host, virus strain, and presence of environmental stressors (Brunner et al. 2005; Forson & Storfor 2006; Schock et al. 2009; Hoverman et al. 2010; Whittington et al. 2010). The impact of ranaviruses on **reptilian** population dynamics and factors contributing to pathogenicity and host susceptibility have been largely unexplored.

In turtles, research has focused on surveillance and isolation of ranavirus from natural populations and reports of related deaths in captive and wild species. Reviews of ranavirus incidence in over a dozen species of **chelonians** globally are provided by Marschang (2011) and McGuire & Miller (2012). Experimental studies are currently underway to examine the importance of virus strain, temperature, and interactions with chemical stressors on susceptibility to and impact of ranavirus in Red-Eared Sliders (Miller & Goodman, unpub. data). In the US, there have been no published reports of ranavirus infection in **crocodilian**s. It was suspected but not confirmed to be associated with death in three juvenile captive alligators (Miller, D.L. Unpublished diagnostic cases from University of Georgia Veterinary Diagnostic and Investigational Laboratory). Ariel (1997) demonstrated that an Australian crocodilian (*Crocodylus johnstoni*) produced serum reactivity to experimental inoculation with BIV. Based on these limited finding, crocodilians should be investigated as possible reservoirs of ranavirus.

Reports of infection in squamates:

To date, all reports of ranavirus in **squamates** have been limited to captive populations. Ranavirus infection has been reported in two families of **snakes** (Pythonidae: *Chondropython viridis*, Hyatt et al. 2002; Viperidae: *Bothrops moojeni*, Johnsrude et al. 1997) and three families of **lizards** (Gekkonidae: *Uroplatus fimbriatus*, Marschang et al. 2005; Chamaeleonidae: *Chamaeleo quadricornis* and *Chamaeleo hoehnelli*, Drury et al. 2002; Lacertidae: *Lacerta monticola*, de Matos et al. 2007) outside of the US. Additionally, Ariel (1997) demonstrated that three Australian species in Colubridae

(*Boiga irregularis*, *Dendrelaphis punctulatus* and *Amphiesma mairii*) produced serum reactivity to experimental inoculation with Bohle Iridovirus (BIV, a ranavirus). Investigations of ranaviruses in wild populations of snakes and lizards in the US have not yet been published but are underway.

Signs of Infection:

Among reptiles, ranavirus infection has been studied most in **chelonians**, in which signs include: lethargy, anorexia, ulcerative stomatitis and yellow-white plaques on the tongue, nasal and ocular discharge, aural abscesses, palpebral and cervical edemas, and necrosis and ulceration of esophagus, stomach, and spleen (Allender et al. 2006; Johnson et al. 2008; Marschang 2011). In **squamates**, where study of ranavirus infection is more limited, signs include: ulceration of the nasal mucosa, ulcerative-necrotizing glossitis, granulomatous lesions in the tongue and liver, enlargement of liver, glomerulosclerosis, and intracytoplasmic inclusions of red blood cells (Drury et al. 2002; Marschang et al. 2005; Marschang 2011). Importantly, turtles can test positive for ranavirus and shed the virus without showing any signs of disease (Goodman et al In Press; Johnson 2006). Also, many signs described above are not limited as responses to ranavirus. Therefore, the aforementioned signs cannot be considered pathognomonic for ranavirus infection, and presence of ranavirus does not necessarily indicate that disease will ensue.

Surveillance and Diagnostic Testing:

To date, rigorous surveys for ranavirus in wild populations of reptiles have only been conducted in chelonians. In infected hosts, ranavirus can be isolated from various organs, including liver, kidney, and spleen, as well as any lesions (e.g., of the oral mucosa or intestines). Samples of these organs and lesions are routinely used to sample for ranavirus presence (Johnson et al. 2007; Gray et al. 2012). However, these samples must be obtained via necropsy and large sample sizes are often necessary to detect ranavirus, which occurs at widely varying prevalences in different populations (<5 to >90%; Gray et al. 2009; Goodman et al. In Press; O'Bryan et al. 2012). Non-lethal sampling methods are preferred for surveillance, and two methods have been used and compared in the past decade. Both oral-cloacal swabbing and skin tissue sampling involve less destructive methods; however, they risk obtaining false positives if virions are present in the saliva or feces or present on the skin surface, but have not actually infected the animal. Gray et al. (2012) conducted a controlled infection study with American bullfrog (*Lithobates catesbeianus*) tadpoles and found false-negative and -positive rates of 20% and 6% for tail samples, and 22% and 12% for external body swabs, using liver samples as the standard to indicate infection. This suggest a similar rate of false negatives for tissue samples and swabbing in amphibians; however a field comparison of sampling methods in reptiles suggested a higher rate of false negatives in swabbing (though a different swabbing was used) relative to tissue sampling. Goodman et al. (In Press) detected ranavirus in 11 out of 63 Eastern Painted turtles (*Chrysemys picta picta*; pooled over three sites) using tail clips (distal portion, 5mm in length), but failed to detect ranavirus in any of 50 turtles using oral-cloacal swabs. Clearly, more research is needed to select the best method for field surveillance of ranavirus in reptiles. Additionally, researchers should expect some false negatives to result from non-lethal sampling. Both tissue sampling and oral-cloacal swabs are recommended, until a direct comparison of effectiveness is made for lizards and snakes.

To prevent potential spread of ranavirus between sampling sites, the following disinfectants must be applied to all equipment, footwear, and other surfaces for at least 1 min at these concentrations or higher: Nolvasan, 0.75%; bleach, 3.0%; and Virkon S, 1.0% (Bryan et al. 2009). Any mud or debris should be scrubbed from surfaces before disinfection.

Tissue samples collected for PCR detection of ranavirus should be either kept frozen without liquid or, in the case of field conditions, stored in 70% ethanol. Methods for PCR detection are described by Gray et al. (2012). Recent studies in reptiles have detected ranavirus using sections of liver, kidney, spleen, and small intestine (Goodman, unpub. data; Gray & Miller, unpub. data). Whole animals that are suspected to have succumbed to ranavirus (deceased or humanely euthanized) should preferably be kept cold, but not frozen, on ice and shipped overnight to a laboratory that can perform wildlife necropsies. If this is not possible, carcasses should be stored in 10% neutral buffered formalin (after removing tissue samples for PCR testing). A list of wildlife disease diagnostic laboratories and instructions for packaging and shipping samples are provided by Miller & Gray (2009).

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Recommended Citation:
Goodman R. 2013. Ranaviruses in Squamates. Southeastern Partners in Amphibian and Reptile Conservation, Disease, Pathogens and Parasites Task Team, Information Sheet #17