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Presence of *Ranavirus* in Green Frogs and Eastern Tiger Salamanders on Long Island, New York

Disease has been implicated as a factor in amphibian population die-offs and declines worldwide (Berger et al. 1998; Blaustein et al. 1994; Brunner et al. 2011; Daszak et al. 2000; Gray et al. 2009a; Green et al. 2010; Kiesecker et al. 2001; Laurance et al. 1996; Muths et al. 2006; Muths and Hero 2010; Russell et al. 2011; Skerratt et al. 2007). One hypothesis for the emergence of diseases in amphibian populations is reduced amphibian immunity associated with increased incidence of natural and anthropogenic stressors. Natural stressors include drying ponds, weather variations, and the presence of predators (Gray et al. 2009a), while the anthropogenic stressors include habitat loss and degradation, invasive species, pollution, agricultural land use, global climate change, and acid rain (Blaustein et al. 1994; Carey et al. 2003; Fellers et al. 2001; Forson and Storfer 2006; Gahl and Calhoun 2010; Gray et al. 2009a; Jaconovich et al. 2005; Kiesecker at al. 2001; Knapp and Matthews 2000 ; Pechmann et al. 1991).

While amphibians are susceptible to many pathogens, viruses belonging to the genus *Ranavirus* appear to be responsible for most amphibian die-offs in North America (Brunner et al. 2011; Collins et al. 2004; Gray et al. 2009a; Jaconovich et al. 2005; Muths et al. 2006; Phillott et al. 2010; Russell et al. 2011). The first catastrophic die-offs from *Ranavirus* in the United States were reported in the mid-1990s and have occurred in all regions of the United States (Brunner et al. 2011; Converse and Green 2005; Green et al. 2002; Jaconovich et al. 1997; Jaconovich et al. 2005; Russell et al. 2011). Furthermore, the number of reported mortality events in the United States from *Ranavirus* is 3 to 4 times greater than that for the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (Muths et al. 2006). A variety of North American species have been affected in these mortality events, including several ranid species (*Lithobates* spp.), ambystomatid species (*Ambystoma* spp.), and plethodontid species (Bollinger et al. 1999; Brunner et al. 2011; Gray et al. 2009a; Gray et al. 2009b; Green et al. 2002; Greer et al. 2005; Jancovich et al. 1997; Jancovich et al. 2005). Undoubtedly, additional die-offs have likely occurred but have gone unnoticed.

In New York State, declines in populations of several amphibian species have been documented in recent years. The tiger salamander, *Ambystoma tigrinum*, once abundant in many wetlands throughout Long Island (Bishop 1941), is now listed as a New York State Endangered Species. Other species, such as the green frog, *Lithobates clamitans*, remain common, and their populations appear to be stable. While declines in green frog populations have not been reported, monitoring mortalities in a common species can be an indicator of potential threats to less common species. The purpose of this paper is to document observed *Ranavirus* presence in mortalities of green frogs and tiger salamanders in a single wetland population at Brookhaven National Laboratory, Long Island, New York in 2007 and 2008 (Due to the endangered status of the eastern tiger salamander in New York, we cannot report the exact locality on the BNL property).

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In 2007, we collected 10 dead pre-metamorphic tiger salamanders at the study location. In 2008, an additional 5 dead juvenile green frogs between Gosner stages 39 and 45 (Gosner 1960) and 4 dead pre-metamorphic tiger salamanders were collected. The mortality event occurred between 6 July – 12 July in 2007, and between 24 June – 3 July in 2008, both periods being very warm and dry (above 82°F during the hottest part of the day for over a week) and occurring just prior to the animals' metamorphosis. Animals that were collected were very bloated, and their ventral surfaces were reddened. Additional carcasses were observed, but were not analyzed because of severe decomposition. We could not estimate the size of the population at this wetland, therefore we cannot provide an estimate of the percentage of the population affected.

We extracted DNA from liver tissue using the DNeasy kit (Qiagen, Valencia, CA, USA). The *Ranavirus* major capsid protein was amplified using the sense primer (5'-GACTTGGCCACTTATCAC -3') and anti-sense primer (5'-GTCTCTGGAGAAGAAGAA-3'), as previously described (Green et al. 2000; Johnson et al. 2008). Using a Taq PCR Kit (New England Biolabs), we amplified mixtures containing the extracted DNA, primers, distilled water, 10x buffer, dNTP, Mg, and Taq in a thermal cycler (PTC-100, MJ Research) with an initial denaturation at 94°C for 2 min., followed by 94°C for 20 sec., 55°C for 30 sec., and 68°C for 2 min. Then after 34 cycles of denaturation at 50°C, the mixture was annealed at 68°C for 7 min. and finally extended at 4°C. PCR products were resolved in 2.0% agarose gels, and bands were examined.

The liver samples confirmed the presence of *Ranavirus* in all nineteen collected specimens from 2007 and 2008. While we cannot conclude that *Ranavirus* caused the mortalities, it is likely that the other carcasses that were not analyzed suffered a similar fate because of the similar appearance, but more advanced decomposition, of those specimens.

The pH of the water at the time of the die-offs in both years was around 10–11, whereas the pH of Pine Barrens ponds on Long Island are generally under 6. Our pond receives storm water runoff from surrounding buildings constructed with Recycled Concrete Aggregate (RCA), which could have elevated the pH observed. It is possible that other runoff from surrounding areas (roadsides, athletic fields, buildings) might have also accumulated in this wetland. These areas are often treated for tick prevention, and salts are used in the winter on icy roads and sidewalks. While we cannot attribute the mortalities to *Ranavirus* specifically, both pesticides and road salt chemicals appear to increase susceptibility to *Ranavirus* in amphibians (Forson and Storfer 2006; Gahl and Calhoun 2010; Gray et al. 2007).

Ranavirus was previously confirmed in eastern box turtles in late 2005 at a pond 2000 m northwest of this location (Johnson et al. 2008), and box turtles were observed consuming carcasses of

larvae that were not able to transform prior to this pond drying (pers. obs.). Larval amphibians were not tested for *Ranavirus* in the nearby ponds because we were not aware of the presence of this disease prior to the observations reported here. Due to the proximity of confirmed *Ranavirus* outbreaks in box turtles, it is possible that the virus was present at our site for some time prior to these events, but was not detected.

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