A RANAVIRUS-RELATED MORTALITY EVENT AND THE FIRST REPORT OF RANAVIRUS IN NEW JERSEY

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Introduction

Although *Ranavirus* has been reported in many states throughout the Northeast, it has until now not been documented in New Jersey. We conducted a side-by-side comparison of PCR and RT-PCR to screen 112 animals from a site in southern New Jersey that experienced a mass mortality event involving *Lithobates clamitans* and *Anaxyrus fowleri* tadpoles. Twenty-four of 114 animals tested positive for Ranavirus with PCR and 32/114 tested positive with RT-PCR, suggesting RT-PCR may be a more effective detection method. Three species were infected at this site: *L. clamitans* (tadpole), *A. fowleri* (tadpole), and *L. sphenocephala* (adult). All animals positive for Ranavirus were symptomatic or dead. We have since documented the presence of Ranavirus, including two additional mass mortality events, in three other counties in New Jersey.

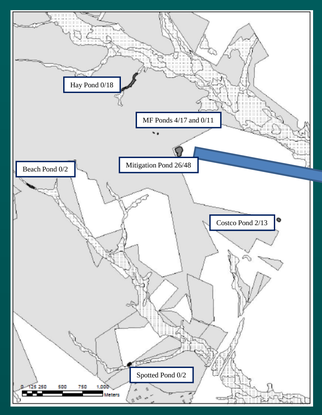


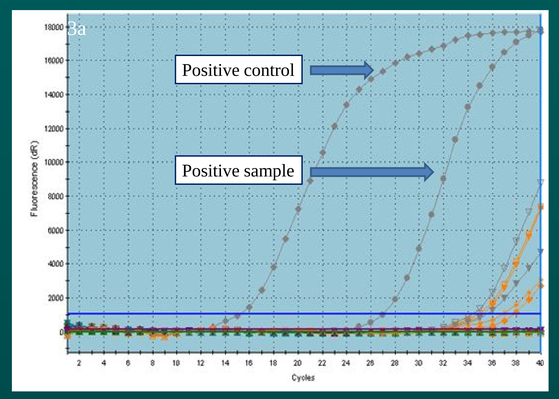
Fig. 1 Map of Ocean County, NJ study sites and positives/sample size

Mitigation Pond, Ocean County, NJ
Photo P. Dolcemascolo

Fig. 2 (a) Symptomatic *L. clamitans* tadpole, (b) *A. fowleri* tadpoles consuming dead *L. clamitans* tadpole, (c) mass mortality of *A. fowleri* tadpoles. All photos P. Dolcemascolo

Lab Methods

We used *Ranavirus*-specific primers MCP4 and MCP5 designed by Mao *et al.* (1997) to amplify approx. 530 bp of the *Ranavirus* major capsid protein gene using both traditional and RT-PCR. For traditional PCR we used 2 µl of DNA in 25 µl reactions that included the following components: 0.4 µM F and R primers, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, and 0.1 U/µl taq polymerase. We ran PCR reactions under the following conditions: 1 cycle of 95°C for 5 min followed by 30 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s, followed by a final 7 min extension at 72°C. Negative controls using water in place of DNA were included with each PCR run. PCR products were checked for the presence of the *Ranavirus*-specific fragment on 2% 1XTAE gels by SYBR Safe (Invitrogen) staining and UV illumination. PCR product for six putative positives was sequenced and compared to known ranavirus major capsid protein DNA sequence. All six samples were identical to each other and shared 99% similarity to an isolate of Frog Virus 3, confirming their identity as a ranavirus. We used RT-PCR to re-screen all samples. We used 4.6 µl of DNA in 10 µl reactions that included the following components: 0.4 µM F and R primers, 5 µl of Brilliant II SYBR Green RT-PCR Master Mix (Agilent Technologies), and H_2O to a final volume of 10 µl. We ran positive control reactions using 2 µl of *L. clamitans* tadpole DNA that tested positive using traditional PCR and negative control reactions under the same conditions. PCR reactions were subjected to 1 cycle of 95°C for 10 min followed by 40 cycles of 95°C for 45 s, 50°C for 30 s, and 72°C for 30 s.



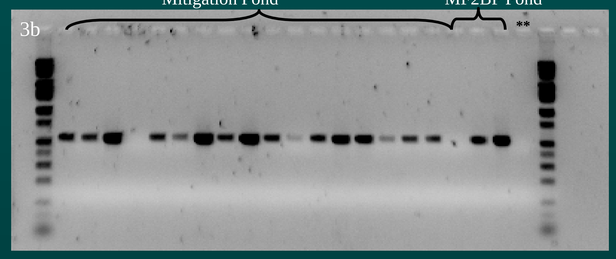


Fig. 3 (a) RT-PCR results of a positive *L. clamitans* tadpole and positive control, (b) Traditional PCR results of *L. clamitans* tadpoles (** negative control)

Fig. 4 Map of New Jersey showing current cases of ranavirus documented by our team

Field Methods

Animals were sampled from five ponds located in Ocean County, NJ, within an area that is being managed for the benefit of pine snake (*Pituophis melanoleucus*) populations (Fig. 1). Sampling occurred on 17 May, 26 May, and 16 June 2011. Two other ponds, Beach Pond and Spotted Pond, were only sampled on 26 May. We retrieved dead and dying *L. clamitans* and *A. fowleri* tadpoles (Fig. 2) by net, stored them on ice for approximately three hours, and then froze them at -20° C for 24 hours before DNA extraction. To determine if water containing asymptomatic *A. fowleri* tadpoles would test positive for *Ranavirus* DNA, we placed apparently healthy (asymptomatic) *A. fowleri* tadpoles in 1.5 ml eppendorf tubes and allowed them to "swim" in the tube for approximately 30 s. Healthy tadpoles were released back to the pond and the water they had been in (approx. 100 µl) was frozen until DNA extraction. We caught adult animals by net or hand and removed one toe using scissors sterilized in 95% EtOH. Toes were stored at room temperature in Drierite desiccant. All adult frogs, with the exception of one dead *L. sphenocephala*, appeared to be healthy and were released at the point of capture. We extracted DNA from tissue and water using the Qiagen QIAamp DNA Mini Kit following the manufacturer's instructions.

Results/Discussion

Of the 112 animals sampled, 24 tested positive for the presence of *Ranavirus* DNA with traditional PCR and an additional eight (total of 32) tested positive with RT-PCR (Fig. 3). The water from all 14 of the asymptomatic *A. fowleri* tadpoles tested negative for *Ranavirus* using traditional PCR, but eight tested positive with RT-PCR suggesting RT-PCR may be best when pathogen concentration is low. Ranaviral DNA was detected in three of the ponds tested, although low sample sizes prevent us from excluding its presence from the others (Fig. 1). To our knowledge, this is the first documented presence of *Ranavirus* in NJ. Our research demonstrates the need to sample during multiple time periods when a ranavirus outbreak is suspected of occurring. Our initial screening of *A. fowleri* tadpoles during the first sampling session, combined with their healthy physical form, gave the appearance they were not infected with *Ranavirus*. We only detected the presence of *Ranavirus* with traditional PCR after 10 days of exposure to known infected individuals in the same pond. Although we detected *Ranavirus* DNA using a non-lethal technique in asymptomatic *A. fowleri* tadpoles, further comparative tests between our non-lethal sampling and tissue sampling will be necessary before we can determine if this non-lethal method is sensitive enough to consistently detect the presence of *Ranavirus*.

New cases of Ranavirus in NJ: We returned on 22 May 2012 and observed symptomatic animals in ponds affected and unaffected in 2011. We found animals that tested positive in all ponds tested. We have also detected *Ranavirus* in *L. sylvaticus* tadpoles in Camden County in 2012 and in Sussex County in 2013 during mass mortality events. Additionally, as part of a statewide screening project we detected *Ranavirus* in an asymptomatic adult *A. fowleri* from Cape May County in 2012 (Fig. 4).

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