

**Spatial distribution of Iridovirus in the Eastern box turtle population at
Brookhaven National Laboratory:
Implications for transmittance based on home range size**

Sarah J. Snyder

Office of Science, Science Undergraduate Laboratory Internship (SULI)

Unity College

Brookhaven National Laboratory

Upton, NY

August 10, 2007

Prepared in partial fulfillment of the requirements of the Office of Science, Department of Energy's Science Undergraduate Laboratory Internship under the direction of Valorie Titus in the Environmental and Waste Management Services Division at Brookhaven National Laboratory.

Participant:

Signature

Research Advisor:

Signature

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ABSTRACT

Spatial distribution of Iridovirus in the Eastern box turtle population at Brookhaven National Laboratory: Implications for transmittance based on home range size. SARAH SNYDER (Unity College, Unity, ME 04988) VALORIE TITUS (Brookhaven National Laboratory, Upton, NY 11973).

There are currently four recognized genera of the icosahedrally symmetric iridoviruses that infect both invertebrates (*Iridovirus* and *Chloriridovirus*) and poikilothermic vertebrates (*Lymphocystivirus* and *Ranavirus*). Ranaviruses have only been documented in a relatively few number of reptiles when compared to the number of viruses that have been documented in amphibians and fish. Recent detection of ranaviruses in five species of chelonians, including a virus outbreak in a population of Eastern box turtles (*Terrapene carolina carolina*) at Brookhaven National Laboratory, is especially alarming. This discovery poses a threat to box turtles in surrounding areas since the species is listed as special concern in the state of New York. This is a continuing study to ascertain the current distribution of infected turtles at Brookhaven National Laboratory. Turtles were sampled during 2006 and 2007 using systematic transect searching. Cloacal and oral samples were collected from each turtle encountered and DNA was isolated from swabs using DNeasy kit protocols. PCR was used to amplify virus DNA and products were subsequently run on 0.8% agarose gels to determine the presence or absence of Ranavirus. Ranavirus was detected in a liver tissue sample and oral swab obtained from one turtle collected during the summer of 2006. This turtle exhibited advanced symptoms of viral infection including an aural abscess and later died. These results preliminarily suggest that swab sampling and PCR testing may not be adequate

methods for detecting ranavirus in pre-symptomatic turtles, yielding falsely negative results from turtles sampled during the early stages of infection. To further explore the potential transmission of the Ranavirus within the box turtle population, determining individual home range size specific to turtles at the study site was necessary.

Radiotransmitters were attached to 5 box turtles inhabiting the area of Ranavirus discovery and their daily movements were recorded for two summers. Geographic Information Systems was used to digitally map turtle movements and estimate home range size by creating minimum convex polygons. Home ranges of individual turtles are not significantly different from one another, varying between 1.8 ha and 8.2 ha, which is comparable to home range sizes found in other studies. Home ranges also grossly overlap which suggests favorable conditions for virus spread, depending on encounter rates and mode of transmission.

INTRODUCTION

Viruses of the family Iridoviridae are characterized by their icosahedral symmetry. These viruses are large and enveloped, with diameters ranging from 125 to 300 nm (Fig. 1). They contain a linear double-stranded DNA genome which may vary from 140 to 303 kilobase pairs. Viruses are replicated within the cytoplasm at morphologically distinct viral assembly sites where they may then be released into the extracellular space by membrane budding. (Bollinger et al., 1999; Jancovich et al., 2003; Westhouse et al., 1996). There are currently four genera of recognized iridoviruses that infect both invertebrates (*Iridovirus* and *Chloriridovirus*) and poikilothermic vertebrates (*Lymphocystivirus* and *Ranavirus*) (Bollinger, 1999). While *Lymphocystivirus* have only been found in freshwater and marine fishes, *Ranavirus* has been isolated from fish, reptiles, and amphibians. Goldfish virus 1-like viruses are also sometimes considered to belong in a separate genera (Daszak et al., 1999).

Ranaviruses have only been documented in a relatively few number of reptiles when compared to the number of viruses that have been documented in amphibians and fish (reviewed by Daszak et al., 1999; reviewed by Hyatt et al., 2000). The majority of reptile ranaviruses have been observed in chelonians, including free-ranging gopher tortoises (*Gopherus polyphemus*), Hermann's tortoises (*Testudo hermanni*) housed together in a zoo in Switzerland, farmed soft-shelled turtles (*Trionyx sinensis*), captive Burmese star tortoises (*Geochelone platynota*), Florida box turtles (*Terrepenne carolina bauri*) and Eastern box turtles (*Terrepenne carolina carolina*) (Chen, Zheng, and Jiang, 1999; De Voe et al., 2004; Johnson et al., in review; Marschang et al., 1999; Westhouse et al., 1996).

Of important note are the multiple observations of iridovirus infections in Eastern box turtles (*T. carolina carolina*). A ranavirus (referred to as turtle virus-3 or TV3) may be responsible for box turtle epizootics as early as 1991. Reexamination of histologic samples from mortality events involving wild box turtles in Georgia in 1991 and captive box turtles in Texas in 1998 has linked previously unexplained deaths to the virus (Johnson et al., in review).

In Venango County, Pennsylvania (USA) 15 of 66 relocated Eastern box turtles were found dead or moribund with palpebral edema, ocular discharge, and fluid draining from the mouth. PCR analysis for the virus major capsid protein gene confirmed the presence of a ranavirus (Johnson et al., in review).

De Voe et al. (2004) investigated a case of iridovirus infection in seven captive Eastern box turtles housed in mixed species enclosures in North Carolina (USA). Clinical signs of infection included cutaneous abscesses, oral erosions or abscessation, and respiratory distress. Additionally, necropsy revealed hyperemic and edematous lungs, splenomegaly, an enlarged yellow liver, and fibrinoid vasculitis in all animals. Viral particles approximately 150 nm in diameter were observed under electron microscopic examination and DNA from isolated virus particles showed an amino acid sequence that was 98% identical to the *Ranavirus* type species (De Voe et al., 2004).

The current investigation focuses specifically on the discovery of an iridovirus infection in two wild box turtles which were found at Brookhaven National Laboratory in Suffolk County, New York (USA) on 2 August 2005. The turtles exhibited ocular discharge and swelling, aural abscesses, and yellow caseous plaques. One turtle died overnight while the other was treated. However, the treated turtle continued to decline

until its death on 1 September 2005. Later histopathology, PCR, and virus isolation confirmed a ranavirus infection (Johnson et al., in review). This finding poses a threat to box turtles in surrounding areas since the species is listed as Special Concern by the New York State Department of Conservation. According to De Voe et al. (2004), “under appropriate environmental or host circumstances, this ranavirus [TV 3] may be capable of causing considerable morbidity and mortality in Eastern box turtles.”

To better understand the transmission of the iridovirus in infected populations it is necessary to accept transmission as a spatially dependent process and assess it as such. There has been no published literature examining the spatial distribution of ranaviruses in populations of wild reptiles so to fully understand the implications of iridovirus transmittance in populations a case study analysis was needed. In investigating iridovirus transmission in Eastern box turtles at Brookhaven National Laboratory the determination of home range, among other parameters, was necessary in order to evaluate the potential spread of the virus within the turtle population at the Laboratory. Geographic Information Systems (GIS) can be an effective tool in investigating disease spread within populations through digitally mapping the non-infected and infected turtle distribution, home range area, and home range overlap (Pfeiffer and Hugh-Jones, 2002).

Three techniques are generally used to study the movements and home ranges of box turtles: the mark-recapture method, thread-trailing, and radiotelemetry. Radiotelemetry provides a reasonably accurate assessment of both habitat use and movement patterns over a long time span (Dodd, 2001). Although exact routes cannot be determined, radiotelemetry is an effective method of determining home range characteristics.

Habitat quality, structure, diversity, and individual preference are all variables that account for variation in size and spatial distribution of home ranges (Dodd, 2001). Range sizes may be larger in areas with unfavorable environmental conditions and poor habitat while densely populated areas may lead to decreased home range size if crowding pressure is high (Stickel, 1950). Due to this variability in the home range of box turtles at different locations, it is necessary to determine home range size specific to the study area in question. To arrive at an accurate home range estimation, turtles may need to be tracked for extended periods of time and mapping must take into account terrain and vegetation. Turtles generally use core areas of their home range for daily activities and venture farther, sometimes very long distances from their home range, on exploratory excursions, feeding forays, and trips to nesting or overwintering areas (Dodd, 2001).

It is clear that home range is a necessary parameter for analyzing potential virus spread, and, with the variability in home range size, individual turtle populations must be monitored and tracked if an accurate home range is to be determined. The iridovirus outbreak discovered in a box turtle population at Brookhaven National Laboratory in Suffolk County, New York may significantly impact this species of Special Concern. Testing turtles for the virus and mapping the distribution of infected and non-infected turtles as well as determining average home range in the area of virus discovery will allow inferences on potential disease spread to be made if transmittance is through animal contact.

MATERIALS AND METHODS

To ascertain the current distribution of infected turtles at Brookhaven National Laboratory, cloacal and oral samples were collected from turtles encountered on the Laboratory property from chance encounter and through systematic transect searching during two consecutive summers, 2006 and 2007 (Fig. 2). Intensive searching was conducted at the pond site where the infected turtles were found in 2005. Several parameters were measured for each captured turtle including mass, carapace and plastron length and width, and carapace height. Turtles were uniquely marked using a standard shell notching system. The location of encounter was noted using a Global Positioning System (GPS) and weather data was recorded. This data may be useful for future population demographic studies.

DNA was then extracted from swabs using the Buccal Swab Spin Protocol for the DNeasy kit (Quiagen, 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 (Johnson et al., in review). Using a Taq PCR Kit (New England Biolabs), mixtures containing the extracted DNA, primers, distilled water, 10x buffer, dNTP, Mg, and Taq were amplified in a thermal cycler (PTC-100, MJ Research) with an initial denaturation at 94°C for 2.5 min., followed by 94°C for 30 sec., 50°C for 30 sec., and 72°C for 30 sec. Then after 34 cycles of denaturation, the mixture was annealed at 72°C for 10 min. and finally extended at 10°C. PCR products were resolved in 0.8% agarose gels and bands were examined.

In order to determine box turtle home range specific to the study site, radiotransmitters were attached to 5 box turtles inhabiting the area of *Ranavirus*

discovery. Transmitters were attached to the carapace and encased using Oatey epoxy putty, which was later colored black to ensure camouflage (Figs. 3-6). Turtles were tracked daily and their location was recorded using a Global Positioning System (GPS) (Figs. 7-8). Weather and vegetation plot data was also collected and habitat preference was analyzed as a component of another study. Geographic Information Systems (GIS), with Hawth's Analysis Tools, was used to map GPS location points of the tracked turtles and to create minimum convex polygons of turtle home range areas.

RESULTS

Ranavirus was only detected in one turtle, which was collected outside the study site area of original virus discovery. This turtle exhibited advanced signs of the virus, including an aural abscess (Fig. 9). The turtle was taken to a rehabilitator and later died. The turtle was dissected and liver tissue and swabs were obtained from the dead specimen (Fig. 10). The liver and oral swab tested positive for *Ranavirus* by producing a band at 500 base pairs but the cloacal swab did not (Fig. 11). All other swab samples collected from 2006 and 2007 tested negative for the virus and did not produce bands.

After plotting encounter location using GIS home range was calculated, using minimum convex polygons, and ranged from 1.746 ha (TF1) to 8.175 ha (TF3) with a mean area of 4.080 ha (Fig. 12). There was no significant difference between home range areas of individual turtles (Chi^2 Value (5.147) < Crit. Value (9.488), $\text{df}=4$, $\alpha=0.05$) or between sexes (T Stat (0.062) < Crit. Value (3.182), $\text{df}=3$, $\alpha=0.05$). Home range areas of turtles were greatly overlapping.

DISCUSSION AND CONCLUSION

Ranavirus is still present in the box turtle population at Brookhaven National Laboratory but was only detected in one turtle sampled during the summer of 2006, which exhibited advanced symptoms of viral infection including an aural abscess. These results preliminarily suggest that swab sampling and PCR testing may not be adequate methods for detecting ranavirus in pre-symptomatic turtles. If this is true, infected turtles sampled may have gone undetected if they were in early stages of infection, yielding falsely negative results. Cloacal swabs have been unsuccessful for virus detection, however, oral swabs may be a useful noninvasive method of testing sick turtles for the disease.

Data from the five radio tracked turtles confirms that box turtles have well defined home ranges that often grossly overlap or are completely superimposed and, generally, individual home ranges of box turtles are stable (Stickel, 1989). Individual preference appears to play a significant role in amount of movement and home range area with one turtle traveling nearly twice the distance and area as any other (TF1). Lack of differences between home range areas occupied by male and female turtles rule out differentiation of home range size based on sex. While some turtles occasionally ventured out of their typical range, all turtles tended to return to preferred core areas.

Results from this study are in agreement with Dodd (2001) who generalized home range of box turtles to be fairly small, varying from 1 ha to 5 ha with a diameter less than 300 m. In contrast, turtles in one relocated Long Island population were reported to have home ranges averaging 9.77 ha while another Long Island population had home ranges averaging 6.77 ha. Both populations are assumed to reside under less than ideal habitat

conditions (Cook, 2004). Turtles in Tennessee had an average home range of 1.88 ha and turtles in Maryland had an average home range of 1.20 ha for males and 1.13 ha for females (Donalson and Echternacht, 2005; Stickel, 1989). These latter two populations are considered to occupy areas of optimal habitat. These findings suggest that habitat at Brookhaven National Laboratory is well suited for Eastern box turtles, eliminating the need for an extensive home range area. Stickel (1950) suggested that maximum travel distances may be recorded over a period of days or weeks while minimum distances may be recorded over months or years.

The overlapping home range areas of box turtles at the study site appear to be in accordance with findings of similar studies. The high degree of overlap of the home ranges is an important factor in the spread of iridovirus. While the virus may be contained in a relatively small area, spread to many individuals is likely. With only 3 confirmed virus infections, spatial mapping and disease modeling based on home range size is not a valuable management tool for controlling disease spread. Further refinement of virus detection techniques and more intensive sampling is needed to determine the extent to which *Ranavirus* may impact the box turtle population.

ACKNOWLEDGMENTS

I would like to thank the Department of Energy and staff at Brookhaven National Laboratory's Office of Science for the opportunity to participate in the Student Undergraduate Laboratory Internship (SULI) Program. I would especially like to send my thanks to my exceptional mentor Valorie Titus, my enthusiastic volunteers Lauren Puccia and Shirin Jaggi, and Danny Kohut for his help in the lab.

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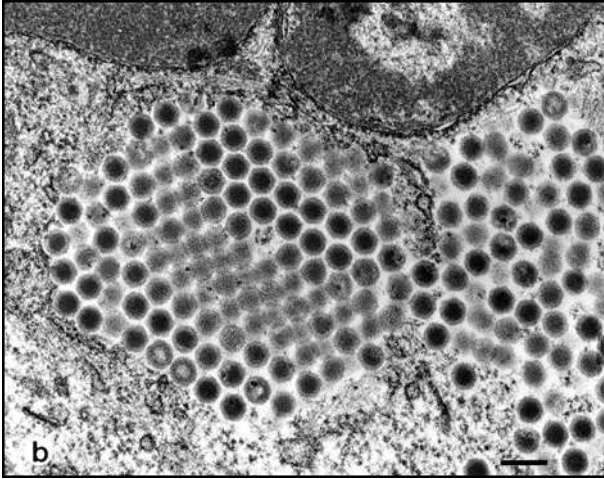
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Figures

Fig. 1- Icosahedral Iridovirus particles



From: <http://www.cdc.gov/ncidod/EID/vol5no6/daszakG4.htm>

Fig. 2- Oral swabbing of a sampled turtle



Figs. 3-6: Applying radiotransmitter to box turtle



Fig. 7- Radiotracking turtles at study site



Fig. 8- Collecting data in the field



Fig. 9- Infected box turtle found with aural abscess Fig. 10- Dissecting infected turtles



Fig. 11- Agarose gel with bands from infected turtles

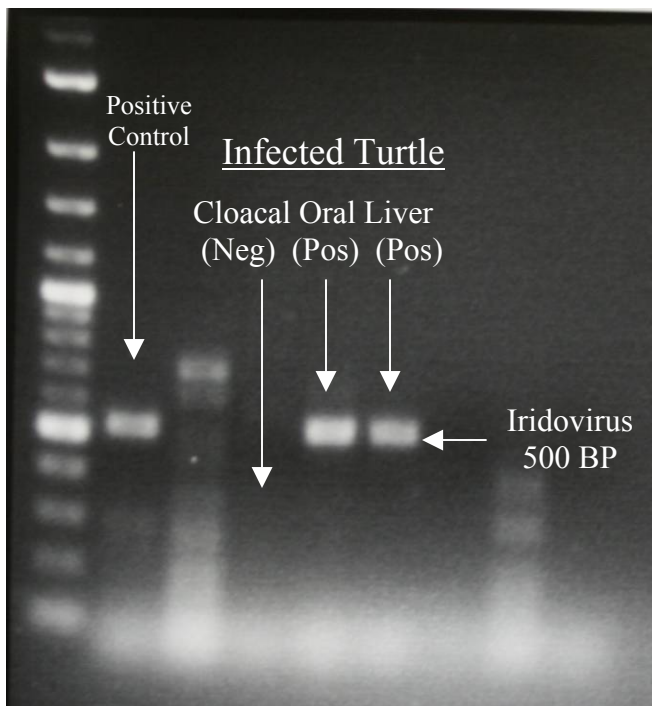


Fig. 12 - Map representing encounter locations and home ranges of radio tracked turtles using minimum convex polygons

