

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

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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 which exhibited advanced symptoms of viral infection including an aural abscess which 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

Iridovirus

Characteristics of Family Iridoviridae

- Icosahedral symmetry
- Large, diameters 125 to 300 nm
- Linear double-stranded DNA 140 to 303 kilobase pairs

Four Genera

- *Iridovirus* and *Chloriridovirus*- invertebrates
- *Lymphocystivirus*- freshwater and marine fishes
- *Ranavirus*- fish, reptiles, and amphibians
- Majority of reptile ranaviruses observed in chelonians

Iridovirus in box turtles

- Multiple observations of iridovirus infections in Eastern box turtles (*Terrapene carolina carolina*) [1,2]
- Two found at Brookhaven National Laboratory on 2 August 2005 with ocular discharge and swelling, aural abscesses, and yellow caseous plaques
- Histopathology, PCR, and virus isolation confirmed a ranavirus infection [1]
- Species is listed as Special Concern in New York

Box turtle home range

- Habitat quality, structure, diversity, and individual preference all account for variation in size and spatial distribution of home ranges [3]
- Home range estimations vary from 1 to 9.77 ha [3,4]
- 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 [5]
- Radiotelemetry useful to assess habitat use and movement patterns over a long time span

Materials and Methods

- Cloacal and oral samples were collected from turtles encountered on the Laboratory property from chance encounter and through systematic transect searching at site where *Ranavirus* was discovered.



- DNA was 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'-GACTTGGCCACTTATC-3') and anti-sense primer (5'-GTCTCTGGAGAAGAAGAA-3') as previously described [1].

- 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. PCR products were resolved in 0.8% agarose gels and bands were examined.



- Radiotransmitters were attached to 5 box turtles inhabiting the area of *Ranavirus* discovery.



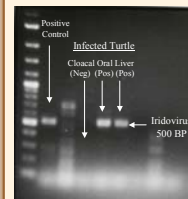
- Turtles were tracked daily and their location was recorded using a GPS. Veg. data was collected.



- GIS was used to map GPS turtle location points and calculate home range area using minimum convex polygons (Hawth's Analysis Tools).

Results- Iridovirus Testing

PCR Products Resolved on Agarose Gel



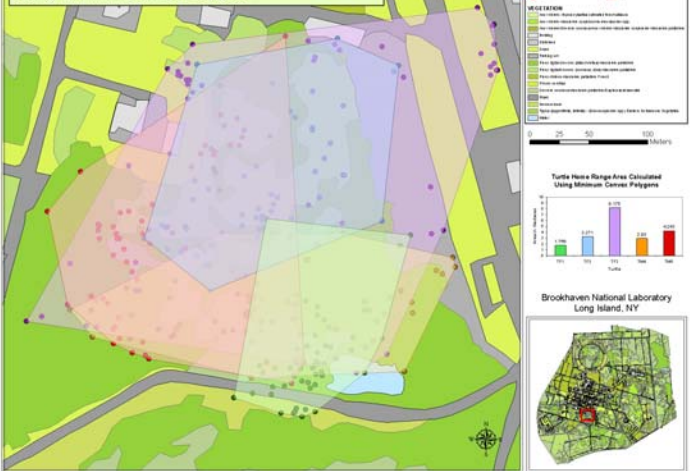
Infected Turtle



Results- Home Range Area

- Min. home range area= 1.756 ha (TF1)
- Max. home range area= 8.175 ha (TF3)
- Mean home range area= 4.08 ha
- No sig. diff. between home range area of individual turtles:
■ Chi² Value (5.147): Crit. Value (9.488), df= 4, $\alpha= 0.05$
- No sig. diff. between home range area of males and females:
■ T Stat (0.062): Crit. Value (3.182), df=3, $\alpha= 0.05$

Distribution and Overlap of Box Turtle Home Ranges Using Minimum Convex Polygons



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. This turtle was collected from a different site than the infected turtles found during the previous summer. The turtle was admitted to a rehabilitation facility and later expired. Liver tissue and oral swab collected from the dead specimen yielded a positive result while the cloacal swab did not detect the presence of the virus.
- 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.
- Home range area of the five radio tracked box turtles ranges from 1.756 ha to 8.175 ha and is consistent with the home range findings of similar studies [3].
- There is no significant difference between the home range area of individual turtles or between sexes, however, one female had a home range nearly twice as large as any other turtle.
- Although home range sizes are relatively small, they grossly overlap as is consistent with the literature which may encourage ranavirus transmission depending on encounter rates and mode or transmission [6].
- 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.

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