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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ABSTRACT

There are currently four recognized genera of the icosohedrally symmetric iridoviruses that infect both invertebrates (*Iridovirus* and *Chlorirdovirus*) 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. Relatively 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. To ascertain the current distribution of infected turtles at Brookhaven National Laboratory, cloacal and oral samples were collected and virus testing was performed using molecular genetic techniques. To further explore the potential transmission of the ranavirus within the box turtle population, determining individual home range size was necessary. Habitat quality, structure, diversity, individual preference, and population density all account for variation in size and spatial structure of box turtle home ranges. Due to this variability, it was crucial to determine home range size specific to the study area in question. Radiotransmitters were attached to 5 box turtles inhabiting the area of *Ranavirus* discovery and their daily movements and habitat preferences were recorded. Geographic Information Systems (GIS) was used to digitally map home range area in order to determine *Ranavirus* dynamics and the potential for disease spread within the box turtle population. Preliminary results indicate that the virus is likely present in the box turtle population at Brookhaven National Laboratory. Home ranges of turtles appear to be relatively small but overlapping 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. 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 extracelluar space by membrane budding [1,2,3]. There are currently four genera of recognized iridoviruses that infect both invertebrates (*Iridovirus* and *Chlorirdovirus*) and poikilothermic vertebrates (*Lymphocystivirus* and *Ranavirus*) [1]. While *Lymphocystivirus* have only been found in freshwater and marine fishes, *Ranavirus* has been isolated from fish, reptiles, and amphibians.

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 [4,5]. The majority of reptile ranaviruses have been observed in chelonians. Of important note are the multiple observations of iridovirus infections in Eastern box turtles (*Terrepene carolina carolina*). A ranavirus (TV3) may be responsible for box turtle epizootics as early as 1991. 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. Later histopathology, PCR, and virus isolation confirmed a ranavirus infection [6]. 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." [7]

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. 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 [8]. Habitat quality, structure, diversity, and individual preference all account for variation in size and spatial distribution of home ranges. This explains the wide array of box turtle home range estimations that vary from 1 to 9.77 ha [8]. Due to this variability, it is necessary to determine home range size specific to the study area in question. 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 [9]. Spatial analysis will allow inferences to be made on potential disease spread if transmittance is through animal contact.

MATERIALS AND METHODS

Iridovirus Testing

•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. Intensive searching was conducted at the pond site where the infected turtles were found in 2005.

•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 [6]. Turtle DNA was also amplified as a control.

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

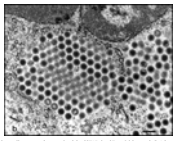
Home Range Analysis

•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.

•Turtles were tracked daily and their location was recorded using a Global Positioning System (GPS). Weather and vegetation plot data was also collected for future analysis of habitat preferences.

•Using Geographic Information Systems (GIS) daily and total movements and minimum convex polygons were used to analyze the home range of individual turtles and to determine average home range and chance of encounter between turtles.

Fig. 1- Icosohedral Iridovirus particles



http://www.cdc.gov/ncidod/EID/vol5no6/daszak/G4.htm

Fig. 2- Box turtle found with aural abscess

Figures 3-6: Applying radiotransmitter to box turtle

Fig. 7- Radiotracking turtles at study site

Fig. 8- Collecting data in the field

RESULTS

Fig. 9- Home Range Area of Tracked Turtles

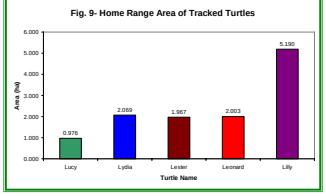


Fig. 10- Average Distance Between Location Points

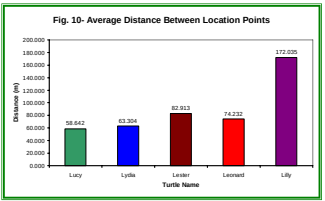
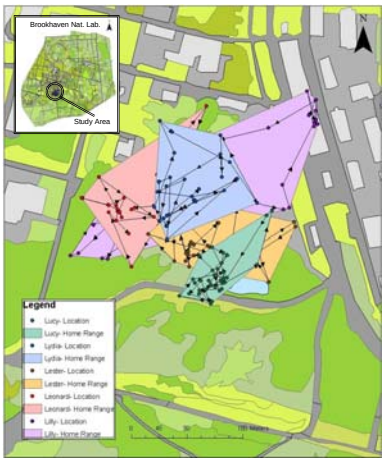


Table 1- Number of times direct paths between locations cross

	Lucy	Lydia	Lester	Leonard	Lilly
Lucy	X	0	9	0	0
Lydia	0	X	4	14	5
Lester	9	4	X	2	0
Leonard	0	14	2	X	39
Lilly	0	5	0	39	X

Fig. 11- Map representing encounter locations, directional movements, and home ranges of radio tracked turtles using minimum convex polygons



We were not able to successfully isolate or amplify either turtle or iridovirus DNA from the oral and cloacal swabs so the distribution of non-infected and infected turtles could not be spatially mapped and analyzed. Two turtles were found during this study (one in the study area) that exhibited viral symptoms including aural abscesses. Both were taken to a rehabilitator and one died shortly after. The abscess on the deceased turtle was tested for turtle and viral DNA but also yielded no results.

DISCUSSION AND CONCLUSION

Preliminary results suggest iridovirus is still present in the population of Eastern box turtles at Brookhaven National Laboratory because two turtles found exhibited advanced signs of infection. The technique used for DNA isolation and amplification is not successful thus far for use with oral and cloacal swabs. Swabbing may not be an adequate means of collecting DNA or the PCR product may have become contaminated. A different thermal cycling regime was followed than was previously described in iridovirus isolation which may also be the source of error.

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 [10]. Analysis of contact using the intersection of direct routes between encounter locations indicates that each turtle may have encountered at least one other transmiterred turtle at least once with some crossing paths almost 40 times. Individual preference appears to play a significant role in amount of movement and home range area with one turtle traveling over twice the distance and area as the others (Lilly) while the other four turtles exhibited similar movement patterns. All turtles tended to return to preferred core areas of their home range. Average distance traveled between encounter locations ranged from 58.642 m (Lucy) to 172.035 m (Lilly) with a mean distance of 90.225 m. Home range calculated using a minimum convex polygon ranged from 0.976 ha (Lucy) to 5.190 ha (Lilly) with a mean area of 2.441 ha. 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 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 [11] suggesting habitat at Brookhaven National Laboratory is well suited for this chelonian.

Although home range of box turtles at the study site appear to be at the smaller end of the spectrum according to the literature, 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. We are not able to test individuals for the virus at this time but it is presumable that, based on the overlapping home ranges of the tracked turtles, the infected turtles that were discovered likely could have spread the disease to turtles within their range. After virus testing techniques are refined, a management plan will be needed in order to evaluate and control the virus in the box turtle population at Brookhaven National Laboratory.

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REFERENCES

[1] Bollinger, T. K., Mao, J., Schock, D., Brigham, R. M., Chinchar, V. G. (1999) Pathology, isolation, and preliminary molecular characterization of a novel iridovirus from tiger salamanders in Saskatchewan. *Journal of Wildlife Diseases*, 35: 413-429.
[2] Jancovich, J. K., Davidson, E. W., Morado, J. F., Jacobs, B. L. Collins, J. P. (1997) Isolation of a lethal virus from the endangered tiger salamander *Ambystoma tigrinum stebbinsi*. *Diseases of Aquatic Organisms*, 31: 161-167.
[3] Westhouse, R. A. Jacobson, E. R., Harris, R. K., Winter, K. R., and Homer, B. L. (1996) Respiratory and Pharyngo-Esophageal Iridovirus Infection in a Gopher Tortoise (*Gopherus polyphemus*). *Journal of Wildlife Diseases*, 32: 682-686.
[4] Daszak, P. et al. (1999) Emerging Infectious Diseases and Amphibian Population Declines. *Emerging Infectious Diseases*, 5.
[5] Hyatt, A. D. et al. (2000) Comparative studies of piscine and amphibian iridoviruses. *Archives of Virology*, 145: 301-331.
[6] Johnson et al. (in review) Ranavirus infection of free-ranging and captive box turtles and tortoises in the United States.
[7] De Voe, R. et al. (2004) Ranavirus-associated morbidity and mortality in a group of captive eastern box turtles (*Terrapene carolina carolina*). *Journal of Zoo and Wildlife Medicine*, 35: 534-543.
[8] Dodd, C. K. Jr. (2001) North American Box Turtles: A Natural History, University of Oklahoma Press, Norman.
[9] Pfeiffer, D. U. and Hugh-Jones, M. (2002) Geographical information systems as a tool in epidemiological assessment and wildlife disease management. *O I E Revue Scientifique et Technique*, 21: 91-102.
[10] Stickel, L. F. (1989) Home Range Behavior Among Box Turtles (*Terrapene c. Carolina*) of a Bottomland Forest in Maryland. *Journal of Herpetology*, 23: 40-44.
[11] Cook, R. P. (2004) Dispersal, home range establishment, survival, and reproduction of translocated eastern box turtles, *Terrapene c. carolina*. *Applied Herpetology*, 1: 197-228.