

Techniques Used in Determining the Presence of the Iridovirus in Samples from Eastern Box Turtles at Brookhaven National Laboratory



STEPHANIE ZITVOGEL¹ and VALORIE TITUS^{2,3}

¹ Babylon Jr. Sr. High School, Babylon, NY 11702

² Brookhaven National Lab, Upton, NY 11973

³ Binghamton University, Binghamton, NY 13903



<http://www.marlabhisa.com/images/homeimages/EasternBoxTurtle.jpg>

ABSTRACT

The populations of fish, amphibians, and reptiles of Brookhaven National Laboratory became threatened by the Iridovirus two years ago, when several sick Eastern box turtles (*Terrapene carolina carolina*) were discovered. It is necessary to continue searching for and testing animals to learn if the virus is still present, and if it is, how far has it spread. The virus has the potential to cause decline of the Brookhaven National Laboratory population of The Eastern box turtles. Tracking the virus in Box Turtles is especially important because in New York, the species has been placed in the category "special concern", meaning Box Turtles are faced with the possibility of becoming endangered. To observe the presence and/or progression of the Iridovirus in turtles, samples were taken from turtles found on BNL property and tested for the virus by means of DNA extraction and PCR. The results of the testing were compared to a positive sampling from an infected turtle.

INTRODUCTION

The Eastern Box Turtle (*Terrapene carolina carolina*) is found only in the United States. The population ranges from Southern Maine to Northern Florida and from the east coast to Illinois, Kentucky, Tennessee, and northeastern Mississippi (see Figure 1). The Box Turtle prefers open, wet woodlands, swamps, and meadows that are near freshwater bodies such as ponds, streams, and even puddles [1,2,3].

Eastern Box Turtles are one of six subspecies constituting the species *Terrapene carolina*. This species can grow up to 9 inches long. Box Turtles' shell, called the carapace, is dark brown with orange-yellow streaks; the carapace is also high-domed with 12-13 scutes on each side. Each of the turtle's 4 legs have 4 clawed toes. Male Box Turtles have bright red-orange eyes and long tails, whereas the females have brownish-yellow eyes and shorter tails. [2]. Box Turtles are omnivorous; they will eat berries, fruit and most types of vegetation, insects, worms and slugs, mushrooms, and sometimes, the remains of dead animals. In the wild, they can live to be 100 years. If the turtle senses danger, it can completely conceal its head, legs, and tail inside its carapace. It does this by pulling the flat underside, called the plastron, up to the edge of the carapace; the turtle is completely invisible once the plastron is up. This serves as an effective defense mechanism and also as a way to easily identify a Box Turtle, as Box Turtles are the only species of turtle that can do this.

The disease now threatening the Box Turtle population of BNL is caused by a virus found in the family Iridoviridae. Iridoviridae contains viruses that infect invertebrates and cold-blooded vertebrates [7]. There are 5 genera in this family of Iridoviruses; the viruses in two of the genera infect insects and other invertebrates. Viruses in the other three infect cold-blooded vertebrates [6]. One of these three genera is *Ranavirus*, which infects some species of fish, amphibians, and reptiles [7]. *Ranavirus* has been discovered in the remains of sick turtles and tortoises, after their tissues were sampled, tested, and analyzed. The symptoms an Iridovirus infection produces in turtles include ocular, oral, and nasal discharge, aural abscesses, lethargy, and anorexia [5,6,7]. Further analysis showed that a likely cause of death was multi-organ failure as a result of the infection produced by the Iridovirus [6].

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<http://www.bio.davidson.edu/people/midorcas/research/Contribute/box%20turtle%20info.htm>

Figure 1: The range of the Box Turtle population (in orange)



Figure 2: Box turtles sampled summer 2008

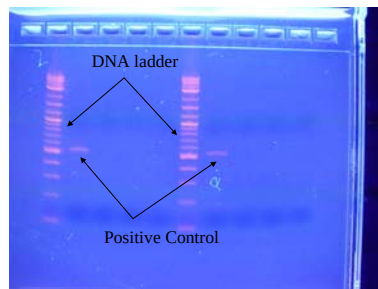


Figure 3: Negative PCR gel from some 2008 turtles.



http://www.wildlife.com/About/Wildlife/NegativeTurtleTissue_images/Box_Turtlehead.jpg

MATERIALS AND METHODS

Eastern Box Turtles prefer to live in wooded areas; they sometimes bury themselves in leaves or hide under bushes, thus camouflaging themselves very effectively. Because of this, they can be very difficult to find. Once found, the turtle is processed; it is marked by filing a notch into its scutes, which are the scale-like structures at the base of the carapace. Each turtle has its own unique set of notches. The ID (notch pattern), date, time, and location are recorded, in addition to the turtle's sex and age. Next, samples are gathered from the turtle; the inside of its mouth and cloaca are swabbed. The swab head is then separated from the stick and placed inside a tube. The temperature in degrees Celsius and humidity are taken and recorded; then the length and width of the turtle's plastron is measured (the turtle must be fully emerged from the carapace in order to get an effective length measurement). The carapace length is also measured, followed by the turtle's mass and a short description of its activities at the time of capture. Lastly, the location of the turtle when it was found is marked with a piece of blue tape, on which is written the turtle's ID and the date. The location is further marked with GPS coordinates.

The DNA contained in the oral and cloacal swabs from the turtles was extracted by using a DNeasy kit (QIAGEN). Once this was done, the extracted DNA was run through a PCR. PCR is a way to stimulate DNA replication at certain points on the strand- this produces many copies of the DNA contained in those points that can be used in testing; in this case, for the Iridovirus. A master mix was added to the DNA extracted from the turtle swabs; this mix is necessary for PCR in that the components actually replicate the DNA. After this was completed, the finished product was separated using a gel plate. Before testing of the swabs was begun, a positive sample from an infected turtle was tested using this same procedure; the positive sampling served as a guide used to compare with the collected turtle samples. Once the finished PCR product completed separation, the plate was observed using ultraviolet light to see if viral DNA was present with the turtle DNA.

RESULTS

Nineteen turtles were sampled and marked in the summer of 2008 (Figure 2). Six out of the nineteen turtles were recaptures from previous years. Oral and cloacal swabs were taken from each of these animals.

After extracting the DNA from the swabs and running it through PCR and gel electrophoresis, and comparing it to the positive sample run using the same procedures, we concluded that Iridovirus DNA was not present in any of the samples from the turtles (Figure 3). The results from the positive testing and the swab extractions proved this conclusion.

DISCUSSION AND CONCLUSION

The DNA bands made visible by gel electrophoresis and UV light clearly didn't match the DNA bands from the positive sampling; this is how we were able to determine if the turtles were infected with the Iridovirus. If any of the turtles had tested positive, the bands of DNA from the turtle samples would have matched that from the positive sample. None of the nineteen samples from this summer tested positive for Iridovirus.

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