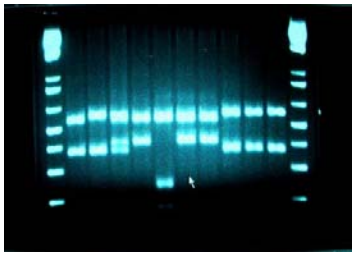
# Genetic Techniques used in Detecting Wildlife Diseases



http://www.oceanexplorer.noaa.gov/explorations/03bio/logs/sept10/media/lasonolide3.html


SHIRIN JAGGI[1] and VALORIE TITUS [2,3]

[1] Hicksville High School, Hicksville, NY 11801

[2] Brookhaven National Lab, Upton, NY 11973

[3] Binghamton University, Binghamton, NY 13903


## ABSTRACT


Iridovirdae, specifically genus Ranavirus, is responsible for the morbidity of frogs and turtles. Alongside, Chytridiomycota, a part of the fungi kingdom, is becoming the cause for the mortality of many amphibians including salamanders and frogs.[1] In order to prevent the spreading of the infection further, it is necessary to find methods to prevent the decline of animal populations. By examining the animal samples collected at Brookhaven National Laboratory, we wish to find genetic methods to detect the cause that is affecting such a large number of animal population so we can prevent the further spread to other animals. We hope to achieve this by using several techniques such as DNA extractions, Polymerase Chain Reaction (PCR), gel electrophoresis and others techniques that are widely used in the field of bioengineering. By finding methods to detect the virus, we hope to see the movement and spread of the virus and consequently find ways to prevent the virus from infecting other animals. As a result, we also wish to figure out how this virus even came to Long Island and how this can affect other animals in the area.


## INTRODUCTION

Iridoviridae, a family of virus with only dsDNA is a genome of 150000-280000 nucleotides long.[2] Having a diameter of 120-300 nanometers and a icosohedral symmetry, the virus consists of five genera: Chloriridovirus, Iridovirus, Lymphocystivirus, Megalocytivirus, and Ranavirus.[3] The virus consists of three domains: an outer proteinaceous capsid, an intermediate lipid membrane, and a central core containing DNA-protein complexes. Usually, *Iridovirus* and *Chloriridoviurs* are known to infect vertebrates which are ectothermic. However this is not known for certain because *Iridovirus* has had recent findings in reptiles as well. *Ranavirus* in specific, can result in high morbidity in susceptible species, such as frogs and toads. [4]

Chytridiomycota, a phylum of the fungi group, are mostly saprobic (degrading chitin and keratin). There are approximately 1,000 chytrid species in 127 genera, which are then distributed among 5 orders. Some chytrid speices are known to kill amphibians in very large numbers. Chytridiomycosis is the disease that is known to fatally infect amphibians caused primarily by the chitrid- *Batrachochytrium dendrobatidis.* This infectious disease is known to have brought dramatic population decline of frogs in western North America, Central America, South America and Australia.[3]

## MATERIALS AND METHODS

In order to carry out genetic techniques and procedures, it was first necessary to obtain animal samples. These samples were obtained by various methods. Eastern Box turtles were found in the woods at Brookhaven National Laboratory, near the area where some turtles were found to be affected with iridovirus. Cloacal and oral swabs were then taken to genetically test the presence of the virus. Two turtles that were found dead and were dissected in order for DNA to be extracted from their liver tissue. Furthermore, tiger salamanders were also tested by cutting part of their tail and tiger salamanders found dead were tested by cutting part of their tongues.

In order to carry out the procedures and test for the presence/absence of any disease, it was necessary to carry out the method of extractions. Extraction was a technique used after all the samples were collected. With this procedure we were able to extract the DNA from the cloacal and oral swabs and tissue samples of the turtles and salamanders. This was done by using a Dneasy kit (Quiagen, Valencia, CA, USA) that provided a protocol for extracting DNA from the various swabs collected and from the tissue samples that were obtained from the sick turtles and salamanders. After the conclusion of the process, DNA was run on an agarose gel just to confirm that the extraction was successful and the DNA was ready for the next step of Polymerase Chain Reaction (PCR).

PCR is used to amplify specific regions of a DNA strand in order to make enough copies that are sufficient to carry out testing. It is first carried out by making a 'master mix' that has the DNA template, which contains the fragment of DNA that needs to be amplified, primers that are complimentary to the DNA regions needed to be amplified, DNA polymerase used to synthesize a DNA copy, deoxynucleotide triphosphate (dNTP's) where the DNA polymerase makes new DNA, a buffer solution that provides a good chemical environment needed for maximum activity and stability of the reaction, and finally magnesium ions.[5] It was during this process that we were able to amplify the DNA to get it ready for the gel electrophoresis, which was to follow.

Gel electrophoresis is the separation of deoxyribonucleic acid, ribonucleic acid and protein using an electric charge.[6] A .8% agarose gel was used to separate the molecules and compare them with the control group. "Electrophoresis" refers to the electromotive force(EMF), which is used to push or pull molecules in a gel by applying an electric current. A blue dye was used to visualize the bands in the gel. A ladder was used during each process of gel electrophoresis.

Figure 1- collecting an oral sample from an Eastern Box Turtle

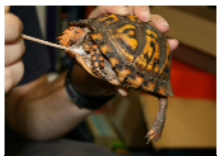
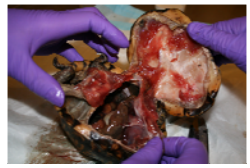


Figure 2- Sick Box Turtle found with an aural abscess

Figure 3- Dissecting a sick turtle to obtain the liver for DNA extraction.



## RESULTS

After the completion of the extractions, performing PCR and running the samples on an agarose gel, it was seen that the sick turtle which exhibited physical characteristics of abnormalty in various regions of its neck, was positive for iridovirus. The positive was only observed in the liver and the oral samples. The other samples obtained from turtles seen from chance encounter and systematic transect searching, did not exhibit iridovirus. Also, the all the tiger salamanders were found negative for Chytrid fungus.

Figure 4- positive liver and oral sample in the sick turtle exhibiting the abscess as compared with the positive Irido sample in the second row,

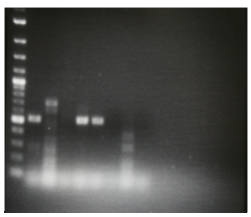


Figure 5- positive Chytrid sample

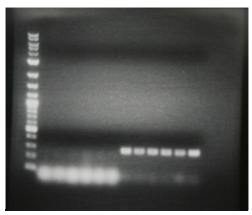
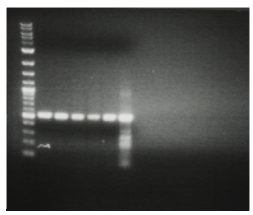


Figure 6- positive Irido sample



## DISCUSSION AND CONCLUSION

According to the gel electrophoresis, there was clearly no iridovirus in the turtles from which cloacal and oral samples were taken. The dead sick turtle that had the aural abscess was positive for Iridovirus. As a result, it is possible to conclude that Iridovirus is positive in turtles that exhibit a physical abnormality as seen in the turtle that was tested. Also, after the DNA was extracted from the oral & cloacal swabs and the liver tissue, the extracted product was run on a gel to test for DNA. The procedure indicated that the swabs and the tissue contained DNA, however when the products were run on PCR and compared with a positive iridovirus sample, only the liver DNA and the oral DNA matched with the positive. To avoid false negatives in the PCR, a positive Iridovirus sample was run on a gel, and it confirmed that there was no iridovirus in the other turtle samples. As a result, it can be inferred that cloacal swabs may not be very effective in determining the presence of Iridovirus.

It was also evident that the sick tiger salamanders found at Brookhaven National Laboratory were not affected by Chytrid Fungus. To exclude the risk of PCR contamination, a Chytrid positive was also run on an agarose gel; the chytrid positive was clearly evident on the gel, confirming that the tiger salamanders did not have the Chytrid fungus.

Although the Chytrid Fungus was not responsible for the death of the several tiger salamanders found in Long Island, it makes one wonder what was the cause of the mortality of the tiger salamanders. Furthermore, how did Iridovirus even come to Long Island. It is necessary to investigate the origins of this virus on Long Island so we can possibly track the virus. If one turtle found on site is affected by Iridovirus, there is a possible risk for other species in the area to be infected as well. Perhaps, we can detect the virus using the spatial distribution of the virus and by using the home range area of the species. Since Brookhaven National Laboratory has a very high population of ticks, it is also necessary to investigate if the virus is transmitted through ticks within the species.

## REFERENCES


[1] Voe, Ryan De., Geissler, K., Elmore, S., Rotstein, D., Lewbart, G., Guy, J.(2004) Ranavirus- Associated Morbidity and Mortality in a Group of Captive Eastern Box Turtles. *Journal of Zoo and Wildlife Medicine*. 35: 534-543

[2] Gopurenko, D., Williams, R.N., McCormick, C.R., DeWoody, J.A.(2006) Insights into the Mating Habits of the Tiger Salamander (*Ambystoma tigrinum tigrinum*) as Revealed by Genetic Parentage Analyses. *Molecular Ecology*, 15: 1917-1928

[3] Williams, R.N., DeWoody, A. (2004) Fluorescent dUTP helps characterize 10 novel tetranuleotide microsatellites from an enriched salamander (*Ambystoma texanum*) Genomic Library. *Molecular Ecology Notes*, 4:17-19

[4] Green, D.E., Converse, K.A., Schrader, A.K.( 2002) Epizootiology of Sixty-Four Amphibian Morbidity and Mortality Events in the USA, 1996-2001. *New York Academy of Sciences*, 969: 323-339

[5] http://users.ugent.be/~avierstr/principles/pcr.html

[6] http://en.wikipedia.org/wiki/Agarose_gel_electrophoresis


## ACKNOWLEDGEMENTS


I would like to thank my mentor Valorie Titus for giving me her immense time and knowledge in assisting me in this project. I would also like to thank Sarah Snyder from the Student Undergraduate Laboratory Internship (SULI) for her time and patience for the genetic lab work. I would also like to thank the Department of Energy and staff at Brookhaven National Laboratory's Office of Science for giving me the opportunity to participate in the High School Research Program (HSRP) and to do research in their facility.


BROOKHAVEN NATIONAL LABORATORY

Office of Science U.S. DEPARTMENT OF ENERGY