A NATURAL HISTORY OF RANAVIRUS IN AN EASTERN BOX TURTLE POPULATION.

Ranavirus is a genus in the family of Iridoviridae. Amphibians and fish had previously been recognized victims of this highly virulent pathogen. In 2003 this virus struck one of two translocated Eastern Box turtle (*Terrapene carolina carolina*) populations that were monitored in adjacent, northwestern Pennsylvania counties. The unaffected habitat, an 80 ha tract in Mercer County, Pennsylvania (PA) and the affected population's habitat was only 20 km away, to the NE in Venango County, PA. Eastern Box turtles have been telemetrically monitored in the Venango County sanctuary for a decade. It is the only chelonian population that has been intensively monitored before, during, and after a Ranavirus outbreak in natural habitat. In 2003-2006 several sudden death events occurred.

On 24 August 2003, one of the Venango County turtles (that had been seen three days earlier to be alert and in apparent good health) was seen near death. The turtle was in poor condition, devoid of muscle tone and had a clear exudate from the mouth. The turtle was brought to an infirmary and began a therapeutic regimen that had been used successfully for respiratory infection. The turtle died 30 h later, on 25 August. The speed with which the turtle died was astonishing. During the 8 weeks spanning late-August to late-October of 2003 this rapid-death scenario repeated 12 times. In all, fifteen turtles (23 % of our population) were apparent victims of the rapid-death syndrome during the 2003 season. All of the turtles that were alive and under soil for winter brumation at the end of 2003 were alive and emerged from the soil at the start (April-May) of the 2004 season. No deaths occurred during the first three months of the 2004 season but on 28 July the first turtle of 2004 died of this syndrome. The disease killed five animals during the latter half of our 2004 season. 2005 repeated the pattern of rapid-deaths starting only after the season's first several disease-free months. The first 2005 case was found on 23 July, five days earlier than the first one in 2004. Three unambiguous cases of the rapid-death disease occurred during the latter half of the 2005 season. Including, in a 2005 census, the year's addition of displaced adults and head-started juveniles to the habitat, these three deaths represented a 4 % population loss for 2005 and a continuation of the declining trend in annual mortality. 2006 suffered only one unambiguous rapid-death (10 July), a juvenile female on the edge of a swamp. This was the earliest of all sudden deaths during the four affected years (2003-2006). A second 2006 sudden-death was an adult male found (2 August) lying at the base of a 10 m cliff. To date (1 October 2009) no sudden-death has occurred since the 10 July 2006 case at our study site. With the annual additions of adults and head-started juveniles since 2006, as part of our long-term repatriation study, the site's population for 2007 was 42 adults and 46 head-started sub-adults; for 2008, 44 adults and 51 head-started sub-adults; for 2009, 51 adults and 55 head-started sub-adults. Of the 24 rapid-death victims, while the disease ran its course from August 2003 to July 2006, 18 (75 %) were on the edge of water (pond, swamp, or seep) when found dead or moribund. Sudden-deaths occurred equally (12 each) between the sexes; 16 victims were juveniles and 8 were adults. When we count all the different adults and juveniles that had lived in this habitat for at least six months during the four year span of 2003-2006, the proportioned mortality rates were 28 % of all juveniles, and 20 % of all adults, exposed to the habitat during this epidemic. Because of our ignorance of the pathogen's means of transmission, contact that each turtle may have had with an infected vector, and other factors that might affect exposure, our simple analysis, suggesting that age-class is not statistically associated ($p = 0.62$) with dying from the pathogen, is not conclusive.

After the first two rapid-death cases in 2003, it was realized that the disease was unfamiliar to the turtle population and so a carcass was sent to various Government and University veterinary diagnostic laboratories in Pennsylvania. The necropsy reports all came back with descriptions of extensive pathology: "severe fibrinonecrotic esophagitis, pneumonitis, acute hepatocellular necrosis, enteritis", "severe acute multifocal ulcerative enteritis and necrotizing splenitis", "congested

lungs with acute hemorrhagic foci", "severe acute necrotizing splenitis, severe suppurative oropharyngitis", "acute pulmonary hemorrhagia and hepatic inflammation". No parasites nor Salmonella were recovered from the moribund or dead turtles. The bacteria that were grown (e.g., *Morganella morganii*; *Streptococcus non-entero* group D; *Streptococcus alpha* hemolytic-L, *Providencia rettgeri*; *Chryseobacterium meningosepticum*-L, *Citrobacter* sp., *Clostridium perfringens*, *Fusobacterium russi*; *Aeromonas hydrophila*, *Stentrophomonas maltophilia*-L, *Vertivillium* sp.-S) varied from turtle to turtle and were evidently opportunists that were secondary to the unknown infection that caused the massive tissue destruction. Toxicology screens of the turtles' organs were all negative.

In frustration of the etiology and biology of the pathogen, late-2003 field precautions were intensified in the hope of minimizing risk of spreading the disease. For example, two volunteers (who had previously helped with tracking) were excluded from the sanctuary so that only two workers would enter the habitat and approach turtles. Clothing and equipment was disinfected with bleach after a session in the habitat. In October 2003, Bob Wagner (University of Pittsburgh's Division of Laboratory Animal Resources) suggested that the laboratory of Elliot Jacobson in Gainesville, at the University of Florida's College of Veterinary Medicine, a leader in uncovering emerging and cryptic reptile pathogens, might be able to shed light on what was decimating our population. After listening to the description of the disease's signs and rapid lethalness, and of local climatic conditions, Elliott speculated that Ranavirus may be the etiologic pathogen. Dead specimens were requested for necropsy and PCR-tests. April Johnson, working in his laboratory, had recently identified Ranavirus-like particles using transmission electron microscopy in archived tissue from unexplained box turtle mass mortality events in Georgia during 1991 and in Texas in 1998. Evidence suggested that this virus was the causative pathogen for those die-offs. Testing our specimens by PCR and virus isolation, she confirmed their suspicion that Ranavirus was killing the box turtles.

In May 2004, April Johnson and April Childress from Jacobson's lab travelled to northwestern Pennsylvania and collected blood samples from almost all turtles living at the affected site. They also collected dead frogs and tadpoles. PCR and virus isolation revealed that the dead anurans had also been infected with Ranavirus. Restriction enzyme analysis of whole genomes of the turtle and frog isolates showed identical restriction patterns, suggesting they were infected with the same virus and so frogs might represent the disease vector for our outbreak. The turtle serology, however, showed that one individual (an aged female) had antibodies against the virus. Our interpretation of their turtle-serology findings is that this virus kills so quickly there is too little time for most victims to launch an effective immune response; but the authors point out other possible explanations for the serology findings, such as short-lived antibody production by sensitized leukocytes, or a slowly developing immune response that might take many months. Amphibians are known to carry Ranavirus, and the virus was identified in dead frogs from our habitat. This study site experienced record-setting rainfall during summer 2003. Elliot Jacobson (University of Florida College of Veterinary Medicine, 29 October 2003) noted that exceptionally wet summers, particularly after summers of drought (exactly the climate circumstance in our habitat in 2003), might generate unusually robust amphibian populations.

Despite the insights provided to us by the laboratory studies of this newly-recognized chelonian disease, we do not yet know how the virus entered and spread through our population, nor why the turtle death toll steadily declined and finally ended. Belzer speculated that perhaps surviving turtles were genetically protected by virally-incompatible cell receptors; or perhaps that vector populations, or the viral abundance in them, steadily declined. Given the long-term monitoring planned for this population, we may be able to gain further insight into the natural history of the disease should it ever return to this habitat.

Beltzer, W. & Seibert, S. (2010). A natural history of Ranavirus in an eastern box turtle population. Turtle and Tortoise Newsletter. In Press.

PCBS IN SEA TURTLES IN THE CANARIES.

Polychlorinated biphenyls (PCBs) are dangerous manufactured pollutants that can be sequestered by a range of animals. PCBs 28, 31, 52, 101, 138, 153, 180 and 209 were measured in tissue from 30 Loggerhead Turtles *Caretta caretta*, 1 Green Turtle *Chelonia mydas* and 1 Leatherback *Dermochelys coriacea* stranded on the Canary Islands to investigate relations between PCBs, lesions and causes of death. Tissues contained higher levels of PCBs than reported from other geographical regions. Sigma PCB concentrations (1980+/-5320 ng g (-1) wet wt.) in the liver of Loggerheads were higher than in the adipose tissue (450+/-1700 ng g (-1) wet wt.). Concentrations of PCB 209 in the liver (1200+/-3120 ng g (-1) wet wt.) of Loggerheads and in the liver (530 ng g (-1) wet wt.) and adipose tissue (500 ng g (-1) wet wt.) of the leatherback were remarkable. Frequency of PCB 209 in the liver (15.5%) and adipose tissue (31%) were equally high. Cachexia was detected in 7 turtles (22%) and septicemia diagnosed in 10 turtles (31%). Statistically, a positive correlation was detected between Sigma PCBs and cachexia. Poor physical condition, cachexia and/or septicaemia could explain the high levels of PCBs detected. However, no histological lesions were exclusively attributed to the effects of PCBs. The most prevalent infections found in the turtle tissue lesions were ulcerative and purulent oesophagitis, purulent dermatitis, necrotizing enteritis and granulomatous pneumonia. The most frequent bacteria found in tissue were *Escherichia coli*, *Staphylococcus* sp. and *Aeromonas* sp. Although immunosupression as a result of PCB pollution is known, among other factors, such as fishing, poor nutritional status and exposure to micro-organisms to cause turtle death, obscure correlates between PCBs and these factors make determination of analysis of exact cause of death difficult. Often analysis of synergistic effects of pollution related stressors is required.

Orós, J., González-Díaz, O.M. & Monagas, P. (2009). High levels of Polychlorinated Biphenyls in tissues of Atlantic turtles stranded in The Canary Islands, Spain. *Chemosphere* **74** (3), 473-478.

GLOBAL AMPHIBIAN EXTINCTION RISK ASSESSMENT FOR THE PANZOOTIC CHYTRID FUNGUS.

Emerging infectious disease has been shown to increase amphibian species loss and any attempts to reduce extinction rates need to squarely confront this challenge. In this report a procedure is developed for identifying amphibian species that are most at risk from the effects of Chytridiomycosis by combining spatial analyses of key host life-history variables with the pathogen's predicted distribution. The technique is applied to the known global diversity of amphibians in order to prioritize species that are most at risk of loss from disease emergence. This risk assessment shows where limited conservation funds are best deployed in order to prevent further loss of species by enabling ex situ amphibian salvage operations and focusing any potential disease mitigation projects. A map of Worldwide potential distribution of areas where Chytrid fungus could develop is drawn.

Approximately one sixth of all known amphibian species fall with their total distributions into regions potentially suitable to Chytrid. The results identify 379 species in which the entire geographic range is, in terms of climate, of high suitability to Chytrid. So far though, little is known about the current infection or population status of most of the 'Top 379'. Perhaps due to the circumstance that many of them occur in regions from where Chytrid has not been recorded only seven of these species are reported to be infected with Chytrid in nature. The report also suggests that this is the result of limited surveillance for disease rather than the occurrence of healthy populations, as at least 42 species of the 'Top 379' have undergone so called 'rapid enigmatic declines' likely caused by the spread of Chytrid and the effect of chytridiomycosis. The report concludes with a discussion on the limitations of the methodology used and how corrective measures could aid better results.

Rödder, D., Kielgast, J., Bielby, J., Schmidtlein, S., Bosch, J., Garner, T.W.J., Veith, M., Walker, S., Fisher, M.C. & Lötters, S. (2009). Global Amphibian Extinction Risk Assessment for the Panzootic Chytrid Fungus. *Diversity* **1**, 52-66.