The Friends of Nachusa Grasslands
2016 Scientific Research Project Grant Report
Due June 30, 2017

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2016 grant amount: $1960

Please answer the following questions with 1- to 2- sentence summaries:

Research Project Topic: Measures of inflammation and respiratory function as markers of health in ornate box turtles

Research Project Purpose: The purpose of our research was to evaluate protein electrophoretograms and blood gas panels as part of a larger project establishing baseline health and disease risks for ornate box turtles at Nachusa.

Research Project Outcomes to date: Presentation at the Nachusa Science Symposium

Describe how the grant funds you have received from the Friends of Nachusa Grasslands have been used in regard to the above topic, purpose, and/or outcomes:

Grant funds were utilized to purchase 1) 50 cartridges for an i-STAT blood gas instrument, 2) 50 protein electrophoresis panels performed at the University of Miami, 3) Charges associated with shipping blood samples to the University of Miami for processing, and 4) Needles, syringes, and heparinized tubes for blood sample collection and storage.

Describe how your project has benefited the work and goals of Nachusa Grasslands:

The Nachusa Grasslands is committed to restoring native prairie grassland and conserving the species which rely upon this habitat. The Friends of Nachusa have taken special interest in the ornate box turtle (*Terrepene ornata ornata*), a state-threatened chelonian which relies heavily upon grassland habitat (such as the Nachusa Grasslands) in order to survive. Previous studies on the ornate box turtle conducted at Nachusa have focused on population characteristics, movement patterns, and behavior. While understanding population size, structure, connectivity, and resource availability/utilization is important for conservation planning, assessing animal health is under-utilized for informing management decisions. Populations challenged by disease, toxins, or genetic abnormalities may not respond in a predictable way to management interventions, and could fail to rebound successfully after perturbation. Furthermore, understanding infectious disease burden is important when considering animal movement to prevent the introduction of novel pathogens to naïve populations. We are conducting a multi-year

longitudinal study of ornate box turtles at Nachusa to determine how environmental, population, and disease factors impact health. The products of this study include a model for population health which will identify the most important drivers of ornate box turtle wellness, the most useful diagnostic tests for health assessment in this species, and provide practical, evidence-based management recommendations for conservation of ornate box turtles at Nachusa and beyond. The goals of this project are aligned with the conservation mission of the Nachusa Grasslands.

The 2016 grant funds we received were utilized during the first year of the larger project described above. To summarize the methods: Ornate box turtles were located within the Orland Track from May 6-9 2016 using a combination of canine and human searches. GPS coordinates, body temperature (via thermography camera), and habitat data were collected at capture. Complete physical examinations were performed by two veterinarians, and abnormalities were recorded. Heart rates were obtained using a Doppler ultrasound probe. A blood sample was collected for hematology, plasma biochemistry, protein electrophoresis, blood gas panel, and quantitative polymerase chain reaction (qPCR) pathogen testing. Swabs of the oral cavity and cloaca were also collected for pathogen testing. Turtles were weighed, sexed, measured, and the shells were notched for permanent identification. Each animal was released at its site of capture. Funding for hematology, biochemistry panels, and pathogen surveillance was obtained through a State Wildlife Grant, while the Friends of Nachusa funded protein electrophoresis and blood gas panels.

For context, all study results will be summarized first, then the protein electrophoresis and blood gas findings will be highlighted. A total of 72 live and two dead box turtles were evaluated (Figure 1). Sixty-five turtles were classified as adults and 7 were juveniles. Over half the animals evaluated were male (N=44), representing a significant male bias (p=0.002). Physical examination abnormalities included shell abnormalities (N=42), missing digits (N=5), missing tail (N=3), and asymmetrical nares (N=2). The shell abnormalities consisted of mild to significant predator damage (N=34), developmental anomalies (N=6), and healed burn injuries (N=2). Two of the shell injuries were fresh, while the rest appeared healed. Examples of shell abnormalities are displayed in Figure 2.
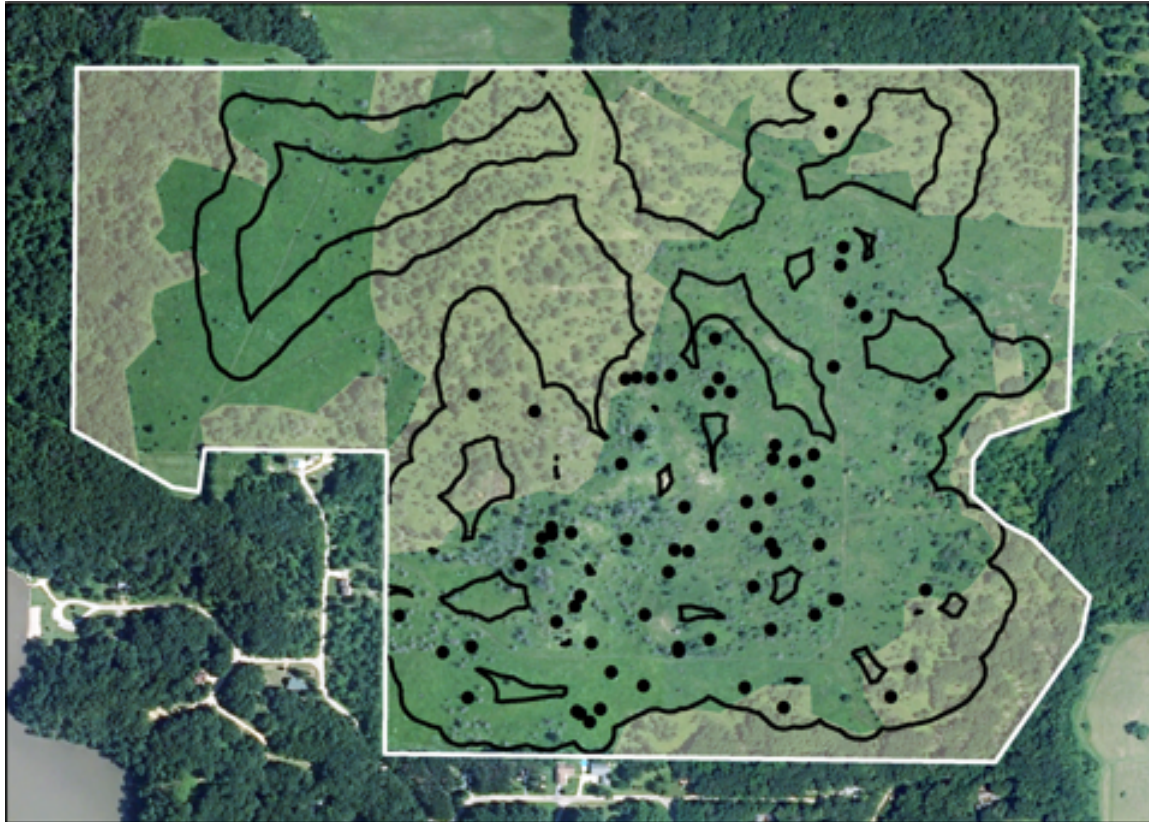
Figure 1. Ornate box turtle capture sites (black circles) and search area (black lines) in the Orland Track of the Nachusa Grasslands during May, 2016.
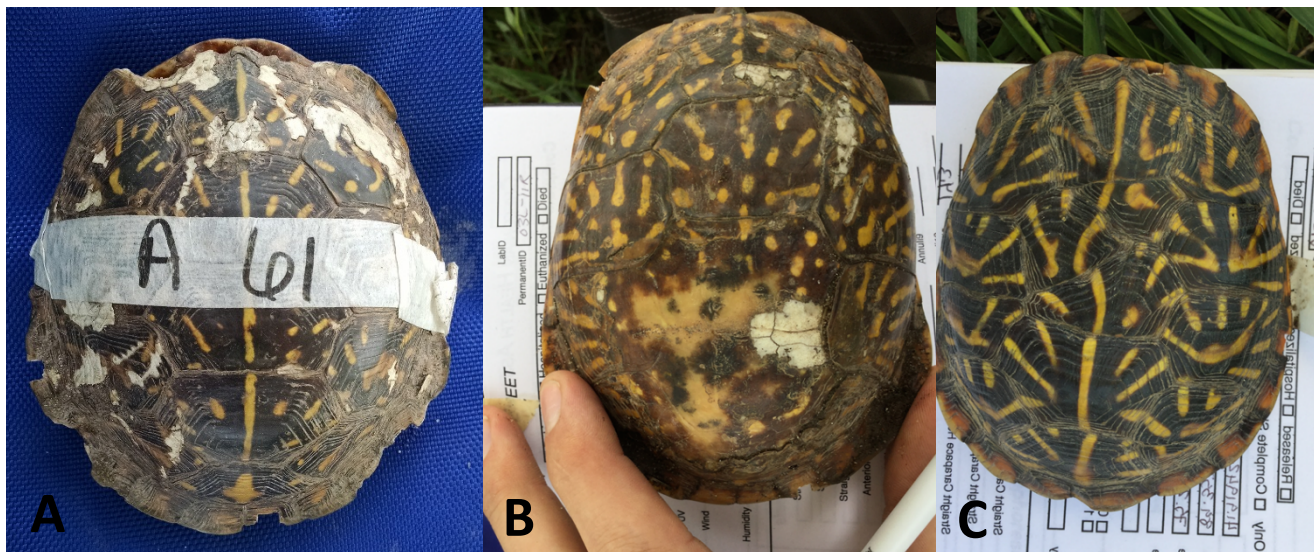

Figure 2. Examples of shell abnormalities identified during physical examination of ornate box turtles at the Nachusa Grasslands in May, 2016. A) Predator damage. B) Healed burn injuries. C) Developmental abnormality of the vertebral scutes.

Hematology (a blood test characterizing the number and distribution of white blood cells, the percent of red blood cells, and the total amount of protein in the blood) was performed in 71 turtles. Plasma biochemistry panels (a blood test for liver function, kidney function, and electrolytes) were performed in 67 turtles. Plasma protein electrophoresis (a blood test describing the distribution of blood proteins) was performed in 63 turtles. Blood gas panels (a blood test for respiratory and metabolic function) were only performed in 46 turtles due to four cartridge errors. Results were generally consistent with those reported for zoo-maintained ornate box turtles and other species of free-living box turtles.

qPCR pathogen testing was performed in all 72 turtles. Terrapene herpesvirus 1 (TerHV1) was detected in 21 turtles, adenovirus was detected in 16 turtles, and TerHV1/adenovirus co-infection was detected in 11 turtles.

BLOOD GAS:

Blood gas panel parameters were dependent upon environmental factors. There were statistically significant associations between the air temperature and three parameters (pCO_2, pH, base excess; $p < 0.05$), the substrate temperature and one parameter (pO_2; $p < 0.05$), the body temperature and three parameters (pCO_2, pH, lactate; $p < 0.05$), and the humidity and four parameters (pH, pO_2, pCO_2, base excess; $p < 0.05$). This is not necessarily surprising, as most blood gas parameters are known to be temperature dependent. There was also a significant association between base excess and heart rate. This is likely related to the metabolic status and subsequent oxygen requirement of the turtle. There were no significant associations between blood gas panel parameters and either physical examination abnormalities or pathogen detection results. Anecdotally, the lactate values of two turtles which were just emerging from brumation were over twice the values of most other turtles. Lactate may therefore be a useful marker of inactivity in box turtles, but it is difficult to state this confidently without replication.

Blood gas panels are extremely useful for assessing respiratory function, circulatory status, and the ability of the kidneys to compensate for metabolic derangement. However, respiratory, metabolic, and organ diseases were infrequently identified in the ornate box turtle population at Nachusa. Instead, traumatic injuries from predators were the most common abnormality identified. Blood gas panel parameters were not predictably affected by the presence of injuries or any of the other physical examination abnormalities identified at Nachusa. Our research therefore indicates that blood gas panels are not a very helpful diagnostic test for assessing the effects of trauma and subclinical infectious diseases in ornate box turtle populations. This information is quite useful moving forward, as it allows us to utilize more effective diagnostic testing options during health assessments.

PROTEIN ELECTROPHORESIS:

In contrast to the blood gas panels, protein electrophoresis panels were affected by injuries and infectious disease status. Turtles with physical examination abnormalities had significantly higher total protein (p=0.01), alpha 2 globulins (p=0.03), beta globulins

(p=0.07), gamma globulins (p=0.003), and a lower albumin/globulin ratio (p=0.02). This is important because it shows that even turtles with healed injuries maintain prolonged inflammatory protein changes. This represents an investment of resources into immune function, and decreases resource allocation to growth and reproduction. Therefore, even if predator injuries do not kill ornate box turtles outright, they result in chronic changes which may affect longevity, growth, and fecundity. Interestingly, few hematologic changes were observed for animals with physical examination abnormalities. The only statistically significant change in abnormal turtles was a decrease in the relative basophil count (p=0.04). Basophil function is poorly understood in reptiles, and the clinical importance of this finding is unknown. Hematology is one of the main diagnostic tests relied upon to assess inflammation and infection in birds and mammals. Our findings suggest that protein electrophoresis may be a more sensitive test to detect the effects of injury in ornate box turtles. We are thankful to the Friends of Nachusa for generously funding another set of protein electrophoretograms in 2017 so that we can continue to explore their diagnostic utility in box turtles.

Turtles with TerHV1 detection had significantly higher total protein (p=0.04), albumin (p=0.006), alpha 2 globulins (p=0.04), and gamma globulins (p=0.006). The same changes plus a significantly higher albumin/globulin ratio (p=0.04) were identified in turtles with adenovirus and TerHV1 co-detection. No significant protein electrophoresis changes were noted for turtles with adenovirus infection alone. On hematology, turtles with adenovirus had decreases in total white blood cell count (p=0.008), lymphocyte count (p=0.005), and an increased relative heterophil count (p=0.03). Turtles with adenovirus/TerHV1 co-detection had the same changes plus a lower relative lymphocyte count (p=0.03). Turtles with TerHV1 infection alone had no significant hematologic changes. These findings suggest that even subclinical pathogen infections result in immunologic responses. They further support the synergistic use of paired hematology and protein electrophoresis panels for characterizing box turtles' response to pathogen infection. Our findings support continued use of hematology and protein electrophoresis panels for health assessment in ornate box turtles.

CONCLUSION:

The Friends of Nachusa allowed us to capitalize on our existing sampling effort in order to evaluate two diagnostic tests in free-living ornate box turtles. One test, protein electrophoresis, proved to be especially useful for assessing the effects of injury and pathogen infection. We now have evidence to support the continued use of protein electrophoresis panels in ornate box turtle population health assessments. Furthermore, we have determined that predator injuries cause significant alteration to box turtle physiology and can make management recommendations (predator control) which may benefit box turtles at Nachusa. These funds have yielded direct results benefitting ornate box turtles, and have successfully supported the work and goals of the Nachusa Grasslands.

Describe how your findings can be applied to challenges in management practices for restoration effectiveness and species of concern:

Our findings are immediately useful because they have identified significant physiologic alteration in box turtles as a result of predator injuries. Instituting predator control measures may benefit the overall wellness of ornate box turtles at Nachusa. Our research has also identified a relatively low disease burden in Nachusa's box turtle population. This could be problematic if foreign box turtles (carrying new diseases) are introduced to Nachusa later on. We therefore recommend infectious disease testing of box turtles prior to introduction. Additional recommendations may be forthcoming following continued health assessment of Nachusa's box turtles in subsequent years.

Please list presentations/posters you have given on your research:

Nachusa Science Symposium, October 22, 2016. "Ornate Box Turtle Health Assessment at Nachusa".

Have you submitted manuscripts to scientific journals? If so, which ones? If not, do you anticipate doing so? (Please keep us informed on publications.**)**

All results obtained in this study will be included in Dr. Adamovicz's thesis. As yet, this data has not been submitted for publication. However, a manuscript on box turtle blood gas panels will be submitted to the Journal of Zoo and Wildlife Medicine shortly.

Optional: Offer suggestions for improving the application and award process for future Friends of Nachusa Grasslands Scientific Research Grants: