

**The Friends of Nachusa Grasslands  
2018 Scientific Research Project Grant Report  
Due June 30, 2019**

1. Please save this form to your desktop with a unique file name that includes “Friends 2018 Science Grant Report” and your last name.
2. Complete the form using the headings in bold as your guide.
3. Save the file as a Word document or a PDF.
4. Attach the file to an e-mail, and send it to: nachusafriendsscience@gmail.com no later than June 30, 2019.
5. The subject of the e-mail should be “2018 Scientific Research Grant Report” and your last name.
6. After your research project is complete, please contact Friends so that we may learn from and publicize the outcomes as appropriate.

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**2018 grant amount:** \$2227

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

**Research Project Topic:** Continued health assessment of ornate box turtles (*Terrapene ornata ornata*) at Nachusa Grasslands through measurement of erythrocyte sedimentation rate, hemoglobin-binding protein, and protein electrophoresis panels.

**Research Project Purpose:** The purpose of our research was to evaluate erythrocyte sedimentation rate, hemoglobin binding protein, and protein electrophoretograms as part of a larger project establishing baseline health and disease risks for ornate box turtles at Nachusa. This information will be used to inform effective management strategies for wild turtle populations.

**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) 65 protein electrophoresis panels performed at the University of Miami, 2) Shipping blood samples to the University of Miami for processing, 3) Commercial kits and control reagents to perform hemoglobin-binding protein and erythrocyte sedimentation rate tests in 50 turtles, 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:**

### Introduction to Our Research and its Place in the Nachusa Grasslands:

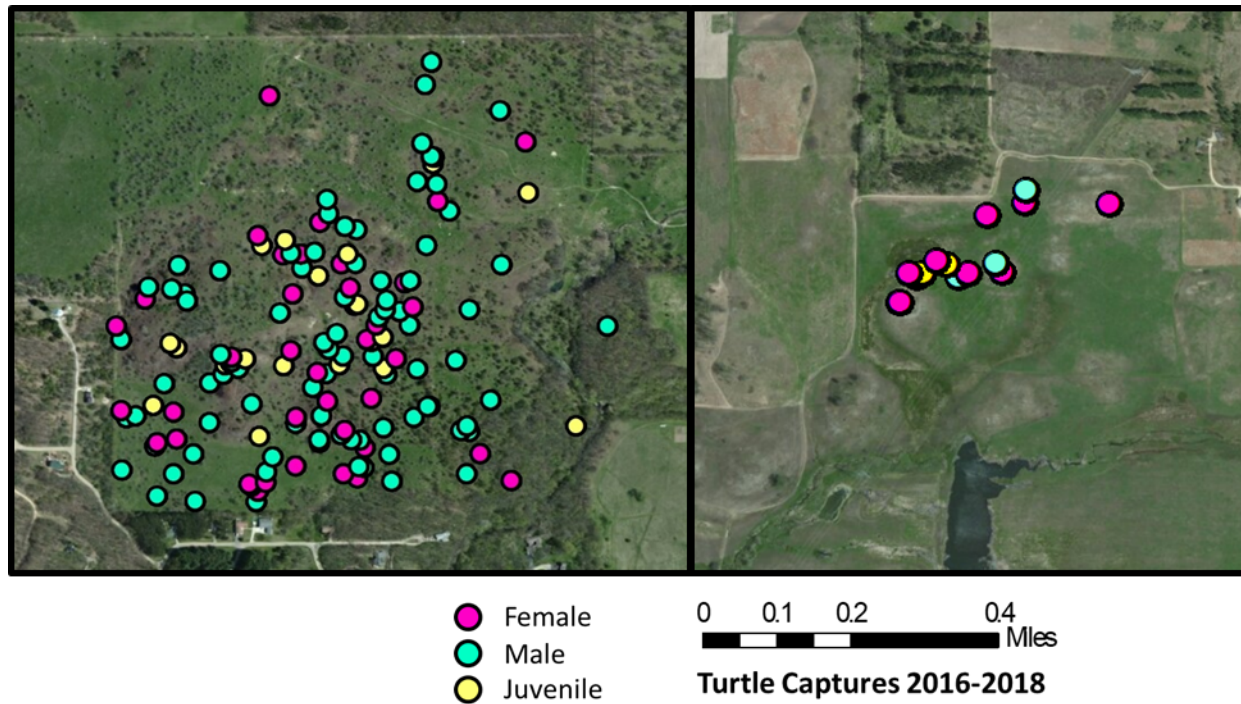
The Nachusa Grasslands is committed to restoring native prairie grassland and conserving the species which rely upon this habitat. The Friends of Nachusa have taken special interest in the ornate box turtle (*Terrepenne ornata ornata*), a state-threatened chelonian which relies heavily upon grassland 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 may also be useful for informing management decisions. Populations challenged by disease, toxins, or genetic abnormalities may not respond in predictable ways to management interventions and could fail to rebound successfully after perturbation. Furthermore, understanding infectious disease burden is important when considering conservation actions like translocations or head-starting (currently under consideration for the box turtles at Nachusa and elsewhere in Lee County) to prevent the introduction of novel pathogens. We are conducting a multi-year longitudinal study of ornate box turtles at Nachusa to determine how temporal, spatial, environmental, and disease factors impact health at both the individual and population levels in order to design more effective conservation strategies. The goals of this project are aligned with the holistic conservation mission of the Nachusa Grasslands.

### Summary of Results from 2016 – 2018:

In 2018, we evaluated sixty-five ornate box turtles at the Nachusa Grasslands from May 7<sup>th</sup>-10<sup>th</sup> including 56 turtles within the Orland Track and nine in the South Bison Unit. Incorporating data from 2016 and 2017, we have now performed a total of 226 health assessments on 168 individual ornate box turtles at Nachusa (Figure 1). Demographic data for live turtles is summarized in Table 1.

Physical examination findings were generally similar between years and sites (Table 1). Shell lesions were the most frequent and striking physical exam abnormality with a prevalence from 51 – 60% each year (Figure 2). These lesions consist of mild to significant predator damage, erosions, flaking, burns, and developmental abnormalities, and many turtles have multiple lesion classifications. To simplify data analysis, we classify each turtle's shell into one of three groups, either within normal limits (WNL), active lesions present (AL), or inactive lesions present (IL). Turtles with both active and inactive shell lesions have abnormalities on multiple traditional blood tests (hematology and chemistry panels) which indicate a significant effect on overall health. However, several other factors including sex, age class, season, and year can also influence the results of these tests, complicating clinical evaluation of health status. To improve our ability to differentiate healthy and unhealthy turtles, we sought to incorporate supplemental diagnostic tests into our routine health assessment protocols.

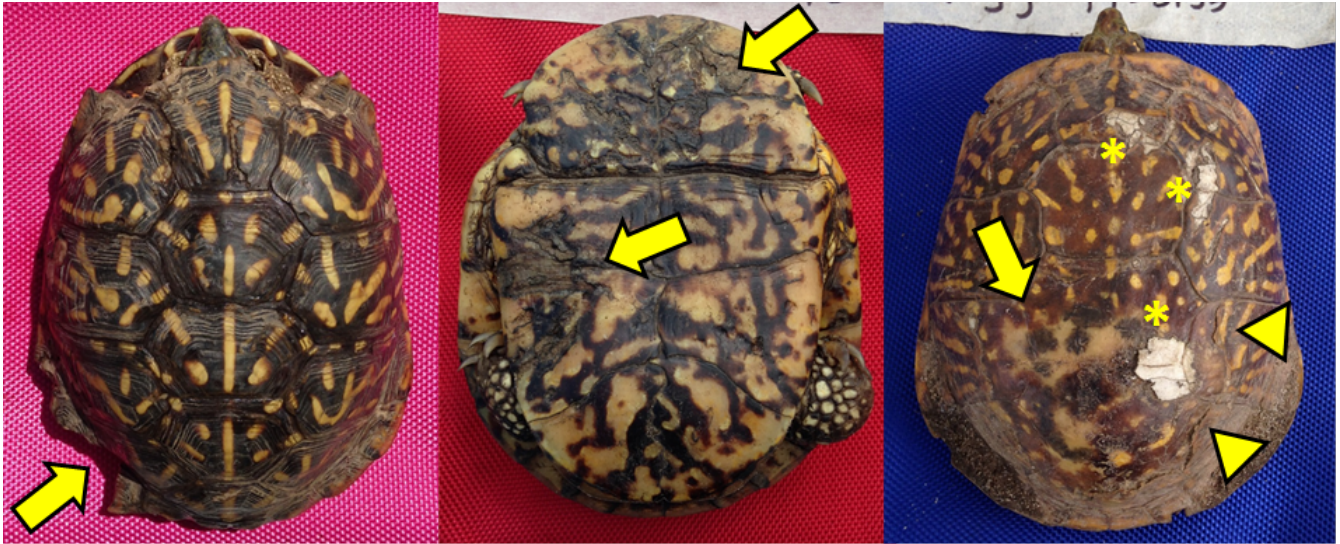
**Figure 1.** Capture locations of ornate box turtles (*Terrapene ornata ornata*) at the Nachusa grasslands from 2016 – 2018. A) Orland Track, B) South Bison Unit.



**Table 1.** Demographic distribution, physical examination findings, and pathogen detection for free-living ornate box turtles (*Terrapene ornata ornata*) sampled at the Nachusa Grasslands in May 2016, 2017, and 2018. TerHV1 = *Terrapene herpesvirus 1*.

		2016	2017	2018
<b>Captures</b>	<b>Total</b>	72	88	65
	<b>Recaptures</b>	0	25	32
<b>Sex</b>	<b>Male</b>	44	41	27
	<b>Female</b>	21	29	37
	<b>Unknown</b>	7	18	1
<b>Age Class</b>	<b>Adult</b>	65	63	63
	<b>Juvenile</b>	7	25	2
<b>Clinical Signs</b>	<b>Eyelid Swelling</b>	0	1 (1%)	0
	<b>Nasal Discharge</b>	0	0	1 (1.5%)
	<b>Asymmetrical Nares</b>	2 (3%)	1 (1%)	1 (1.5%)
	<b>Missing Extremities</b>	8 (11%)	5 (6%)	1 (1.5%)
	<b>Inactive Shell Lesion</b>	34 (47%)	29 (33%)	20 (31%)
	<b>Active Shell Lesion</b>	8 (11%)	19 (22%)	19 (29%)
<b>Pathogens</b>	<b>Adenovirus</b>	16 (22%)	1 (1%)	1 (1.5%)
	<b>TerHV1</b>	21 (30%)	0	0

**Figure 2.** Common physical examination abnormalities identified in free-living ornate box turtles at the Nachusa Grasslands. A) Predator injuries to the marginal scutes, B) Erosions of the plastron, C) Healed burn injury (arrow), bone exposure (asterisks), flaking (arrowhead).



Funding from the Friends of Nachusa has enabled us to perform several supplemental diagnostic tests for health assessment over the last three years, including 163 protein electrophoresis panels (EPH), 215 hemoglobin-binding protein (HBP) tests, and 62 erythrocyte sedimentation rate (ESR) tests. Through another funding source, we were also able to perform 43 ESR tests in another population of ornate box turtles at Ayers sand prairie for comparison. Overall, we have found that each test is useful for supplemental characterization of health status in this species, and we are the first researchers to describe the use of these diagnostic tests in ornate box turtles.

Considering all three years of protein electrophoresis results together, we found that turtles with no shell damage had higher relative albumin concentrations and higher albumin : globulin ratios than animals with both inactive shell lesions ( $p = 0.0001$ ) and active shell lesions ( $p = 0.01$ ). This pattern of changes is consistent with tissue damage and inflammation in turtles with shell damage. Interestingly, these changes are also present in turtles with healed shell damage, reflecting prolonged physiologic changes that persist after clinical resolution of shell injury. This likely represents a diversion of resources away from growth and reproduction and towards wound healing, a shift that may temporarily or permanently affect fitness, fecundity, and survival. These findings support the results of our standard diagnostic tests, and illustrate that protein electrophoresis has a clear place in ornate box turtle health assessment.

Hemoglobin-binding protein and erythrocyte sedimentation rate both demonstrated significant elevations for turtles with acute injuries ( $p < 0.05$ ), but no difference was detected between turtles with inactive shell lesions and those with

completely normal shells. This indicates that these two diagnostic tests have more utility during active disease processes.

The Friends of Nachusa allowed us to capitalize on our existing sampling effort to gather more protein electrophoresis and hemoglobin-binding protein data and to evaluate a new diagnostic test in free-living ornate box turtles. The combination of hematology and protein electrophoresis appears useful for assessment of acute and chronic inflammation in ornate box turtles, while hemoglobin-binding protein and erythrocyte sedimentation rate are more useful for detecting acute injuries and inflammatory disease processes. We have provided another year of objective evidence demonstrating that predator injuries to the shell cause significant alteration to box turtle physiology. As a result, we 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:**

1. Wildlife Disease Association 67th International Conference, August 4-10, 2018. "Modeling Approaches for Promoting Health in Free-Living Ornate Box Turtles (*Terrapene ornata ornata*)"
2. Nachusa Science Symposium, October 20, 2018. "Modeling Health in Ornate Box Turtles (*Terrapene ornata ornata*) at Nachusa"
3. Adamovicz Doctoral Dissertation Defense, November 28, 2018. "The Wellness of Wildlife: Modeling Health in Free-Living Herptiles"
4. Joint meeting of the American Association of Zoo Veterinarians and the Association of Reptile and Amphibian Veterinarians, September 29 – October 3, 2019 (presentation accepted). "Erythrocyte Sedimentation Rate and Hemoglobin-Binding Protein in Eastern and Ornate Box Turtles (*Terrapene* spp.)"

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

We will be submitting a manuscript on erythrocyte sedimentation rate and hemoglobin-binding protein in eastern and ornate box turtles to Ecohealth this month.

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