

A comparative survey of *Gopherus polyphemus* hemoparasites in two differing South Florida habitats

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DISTINCTION THROUGH DISCOVERY

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Abstract

The gopher tortoise (Gopherus polyphemus) is a keystone species which affects the biodiversity that shares its burrow. Habitat fragmentation has led to a decline in populations within southeastern ranges. Haemogregarina (intracellular parasites) have been identified in the blood of gopher tortoises, possibly due to the lack of prescribed fires which are known to kill ticks. However, little is known about their health significance. Two study sites, Blazing Star Preserve (BSP), and Pine Jog Preserve (PIP), differ in their management. The goal of this study is: (1) determine, if any, haemogregarina parasitemia levels of the gopher tortoise population in each site and (2) detect any additional hemoparasite species. We hypothesize that paristemia will be higher in BSP due to increased overcrowding and lack of prescribed fires. This study will benefit current conservation and management practices for gopher tortoises and provide a baseline study for intracellular parasites.

Introduction

Gopher tortoise (Gopherus polyphemus)

- Keystone species
- · Range: Southeastern United States
- · Threatened/Endangered
- Few studies have observed hemoparasites in the gopher tortoise

Gopher Tortoise



Hemoparasites have been found in gopher tortoises but health impact is unknown

Hemogregarine infected erythrocytes.



Figure 1. Light microscopy shows intracellular bodies at 1000x within G. polyphemus erythrocytes at Florida Atlantic University Preserve (FAUP), (Elhassani 2014 unpublished).

Ticks are possible vector for hemoparsites in gopher tortoise

- Fire controls numbers of ectoparasitic arthropods such as ticks [1,2]
- Ticks harbor pathogens which they may transmit to tortoises [3]
- Haemogregarina were first observed in a subset of tortoises in Georgia [4]
- Elevated tick loads may increase the risk of hemoparasite transmission in the gopher tortoise

Hypothesis

Gopher tortoises in Blazing Star Preserve will possess higher hemoparasitemia, when compared to Pine Jog Preserve, due to overcrowding and lack of prescribed fires.

Objectives

Objective 1

Survey two additional south Florida habitats for Haemogregarina parasitemia levels

Objective 2

Identify any additional hemoparasites in the gopher tortoises

Methods

Sites Sampled in this Study

Blazing Star Preserve (BSP) Boca Raton, FL



No prescribed fires – 26 acres

Pine Jog Preserve (PJP) West Palm Beach, FL



Prescribed fires - 160 acres

Field blood drawing procedure



blood drawn from the gopher tortoise



Blood placed in heparincoated vacutainer then stored on ice in cooler



Blood smear stained with Wright-Giemsa and parasitemia was counted (40 separate views under 1000x)

Preliminary Results

Objective 1

Haemogregarina Parasitemia levels in G. polyphemus

- Haemogregarines have been identified in G. polyphemus populations at FAUP
- Parasitemia levels for haemogregarines in G. polyphemus at PJP and BSP are currently being collected

Objective 2

Light microscopy shows cytoplasmic inclusions and potential haemogregarine gametocye of G. polyphemus erythrocytes at both BSP and PJP

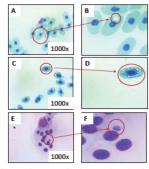


Figure 2: Possible Intracellular inclusion from BSP (A and B). Possible virus inclusion found in both PJP and BSP (C and D) needs PCR

Discussion

- Objective 1: Data is still currently being collected from both G. polyphemus populations.
- Objective 2: Our analysis of blood slides via Wright-Giernsa stains revealed potential virus inclusions which will require PCR using viral primers to determine their identity
- Large potential viral inclusions were observed in both BSP and PJP
 G. polyphemus erythrocytes (A and B). PCR using common reptile virus primers will determine presence of any viruses.
- Multiple G. polyphemus erythrocytes (C and D) displayed inclusions surrounding the nucleus possibly representing the existence of herpes virus. [5] PCR confirmation will be performed to identify existence using appropriate primers.
- Evidence of possible intracellular parasitic protozoan have been observed within G. polyphemus erythrocyte at BSP (E and F). We will use PCR to confirm species of this probable intracellular protozoan.

Future work

- Sample gopher tortoises at other fragmented urbanized sites in South Florida
- Perform PCR on blood samples from the sites at FAUP, JDSP, PJP, and BSP to determine the existence of inclusions
- Re-sample the same tortoises at different time periods when tortoises are mating (May-July) and when adult ticks are feeding (August-October)
- Determine whether intracellular parasitic protozoan are being transmitted by ticks on gopher tortoises
- Test innate immune response of G. polyphemus heterophils to measure direct measure of how their immune system is functioning.

References

- Cully, J.R., J.F. 1999. Lone star tick abundance, fire, and bison grazing in tall-grass prairie. Journal of Range Management 52 139-144
- Hoch, A.L., P.J. Semtner, R.W. Barker, AND J.A. Hair. (1972). Preliminary observations on controlled burning for Lone Star tick (Acarina; Ixodidae) control in woodlots. Journal of Medical Entomology 9 446-451.
- 3 Bari, A. (2013). Analysis of the gopher tortoise tick and its distribution in a small conservation are in Southeasterr Florida. Thesis Manuscript submitted to the Biology Honors Thesis Program
- Hernandez, S.M. et al. (2010). Health and reproductive assessment of a free-ranged gopher tortoise (Gopherus polyphemus) population following translocation. Journal of Herpetological Medicine and Surgery 20(2-3) 84-93.
 Murray, P. R., Rosenthal, K. S., M. A. Pfaller, 2013. Medical Microbiology 7th Ed. Elsevis Saunders

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