Host factors associated with blood parasite infections in aquatic turtles in Georgia

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INTRODUCTION

- ***Haemogregarina,*** **one of the four genera of haemogregarines, are common intraerythrocytic protozoan parasites of reptiles (Telford, 2009).**
- ***Heamogregarina*** **are presumed to be transmitted by leeches based on a single experimental study (Siddall and Desser, 2001).**
- **The behavior of aquatic turtles, along with other factors (e.g. gender, habitat, seasonality, etc.) likely influences the frequency of which turtles come into contact with leeches.**
- **It has been hypothesized that basking behavior of turtles can alter exposure to leeches and subsequence infection of turtles with haemogregarines**
  - **McAuliffe (1977) reported that parasitemias of the nonbasking Common Snapping turtles (*Chelydra serpentina*) was higher than that of two basking species (Eastern Painted turtles (*Chrysemys picta*) and Blanding's turtles (*Emydoidea blandingii*)) in Nebraska.**
  - **Additionally, Davis and Sterrett (2011) reported a similar finding for two basking species, pond slider (*Trachemys scripta*) and *C. picta*, compared with the nonbasking species, the common musk turtle (*Sternotherus odoratus*). However, sample sizes were small (n=12 total for all three species)**

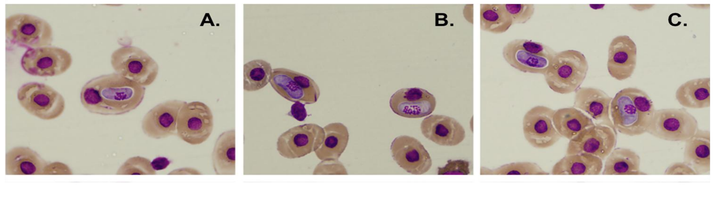
Goals and Hypotheses

The purpose of this study is to expand on previous work and examine differences in haemogregarine prevalence and parastemias among common turtle species from several difference habitats in Georgia and relate any differences to habitat, basking behavior, age, or gender of the turtles.

We hypothesize that non-basking turtles will have an increased chance of infection. Additionally, we hypothesize that different genetic variants of the parasite will be associated with turtle species or turtle family or geographic location.

METHODS

- **Hoop traps baited with sardines were used to capture turtles in several locations in Clarke, Madison, and Baker Counties. Archived blood samples from Costa Rica were included in the molecular aspect of project.**
- **Turtles were marked on their marginal scutes and measurements of their body mass, carapace and plastron were taken. Leeches were counted and a representative sample collected and stored in 70% ethanol for future identification.**
- **Blood samples were collected from the subcarapacial sinus or lateral tail vein.**
- **Blood smears were fixed with methanol and stained with Giemsa.**
- **Blood smears were analyzed to determine haemogregarine infection status and parasitemias were based on number of infected cells per 7,000 erythrocytes examined under a light microscope.**
- **DNA was extracted from the blood sample using DNeasy ® Kit. An ~600 bp region of 18S rRNA gene was amplified with primers Kim18Sf and KimRev2.**
- **Samples were sequenced at the UGA's sequencing facility. Chromatograms were analyzed in Sequencher.**

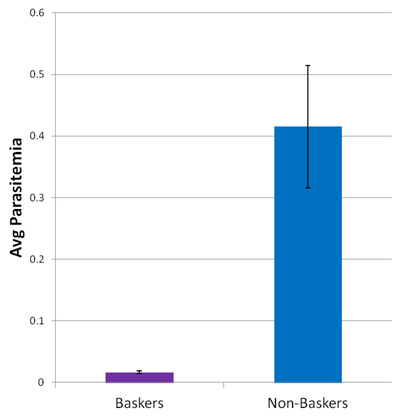


RESULTS

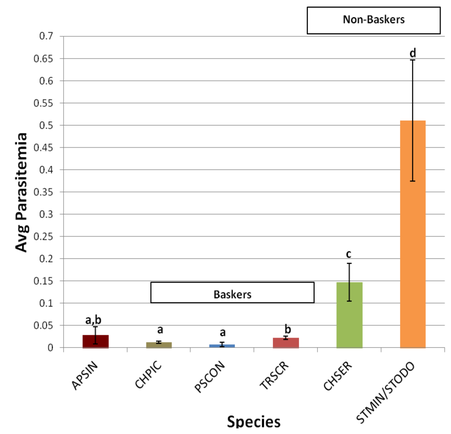
Non-baskers have a higher prevalence compared with baskers; however, gender have no major difference

Group	No. positive/No. tested (%)
Basking	123/286 (43)[a]
Nonbasking	91/103 (88)[b]
Female	83/159 (52)
Male	119/214 (56)
Softshell (ASPIN)	3/4 (75)[a,b,c]
Painted (CHPIC)	49/143 (34)[a]
River cooter (PSCON)	4/16 (25)[a]
Pond slider (TRSCR)	70/127 (55)[b]
Common snapper (CHSER)	12/27 (44)[a,b]
Musk turtles (STODO/STMIN)	70/76 (92)[c]

Parasitemias for nonbasking species was significantly higher than basking species. Among the nonbaskers, CHSER had lower parasitemas compared with STMIN/STODO. Among the baskers, TRSCR had higher parasitemias compared with PSCON and CHPIC.

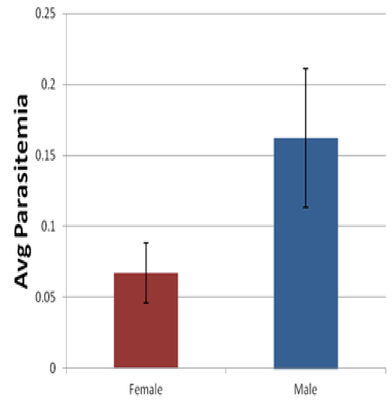


t-Test: p=0.0001



Different letters indicate significant difference

Males had higher parasitemias compared to females, but wasn't significantly higher.



t-Test: p=0.074

Group	Species (ID), Location	32	91	133	202	236	238	241	242	243	346	408	409	423	433	434	440	453	470	519	523
	TRSCR (AQT49), GA	T	T	T	T	A	A	A	T	T	C	T	C	C	C	C	A	T	A	T	C
	CHSER (40AA), GA	T	T	T	T	A	A	A	T	T	C	~	~	~	~	~	~	~	~	~	~
	DERET (AQT2), GA	T	T	T	T	A	A	A	T	T	C	T	C	C	C	C	A	T	A	T	C
	GRBAR (AQT9), GA	T	T	T	T	A	A	A	T	T	C	T	C	C	C	C	A	T	A	T	C
Group A	CHPIC (T37), GA	T	T	T	T	A	A	A	T	T	C	T	C	C	C	C	A	T	A	T	C
	CHPIC (T41), GA	T	T	T	T	A	A	A	T	T	C	T	C	C	C	C	A	T	A	T	C
	CHSER (SCR15), GA	T	T	T	T	A	A	A	T	T	C	T	C	C	C	C	A	T	A	T	C
	BR (C2), Costa Rica	T	T	T	T	A	A	A	T	T	C	T	C	C	C	C	A	T	A	T	C
	STODO (1000), GA	T	T	T	T	A	A	A	T	T	C	T	C	C	C	C	A	T	A	~	~
	MUD (BM2), Costa Rica	T	T	T	T	A	A	A	T	T	C	T	C	C	C	C	A	T	A	~	~
	PSCON (113AA), GA	T	T	T	T	G	G	A	A	A	C	T	C	C	C	C	A	T	G	T	C
	TRSCRIP (T24), GA	T	T	T	T	G	G	A	A	A	C	T	C	C	C	C	A	T	G	T	C
	CHPIC (T35), GA	T	T	T	T	G	G	A	A	A	C	T	C	C	C	C	A	T	G	T	C
Group B	STMIN (4002), GA	T	T	T	T	G	G	A	A	A	C	T	C	C	C	C	A	T	G	T	C
	TRSCR (96 R27), GA	T	T	T	T	G	G	A	A	A	C	~	~	~	~	~	~	~	~	~	~
	CHSER (SCR100), GA	T	T	T	T	G	R	G	A	A	C	T	C	C	C	C	A	T	G	T	C
Group C	CHSER (HQ224959), Canada	C	T	G	A	G	T	T	T	A	T	T	T	T	T	T	G	C	G	G	T
	STMIN (AQT3, GA	C	T	G	A	G	T	T	T	A	T	T	T	C	T	T	G	T	G	G	T
Group D	STMIN (4001), GA	C	T	G	A	G	T	T	T	A	T	T	T	C	T	T	G	T	G	G	T
	STMIN (1206), GA	C	T	G	A	G	T	T	T	A	T	T	T	C	T	T	G	T	G	G	T
	STMIN (1181), GA	C	T	G	A	G	T	T	T	A	T	T	T	C	T	T	G	T	G	G	T
	TRSCR (95 R27), GA	Y	T	G	A	G	K	T	T	A	T	~	~	~	~	~	~	~	~	~	~
Group ?	KISUB (AQT16), GA	T	K	T	T	G	G	A	G	A	C	W	C	C	C	C	A	T	~	~	~
Groups A/B	PSCON (T10), GA	T	T	T	T	R	R	A	A	W	C	T	C	C	C	C	A	T	G	T	C
	TRSCRIP (AQT48), GA	T	T	T	T	R	R	A	A	W	C	T	C	C	C	C	A	T	R	T	C
	TRSCRIP (AQT5), GA	T	T	T	T	R	R	A	T	W	Y	T	C	C	C	C	A	T	R	T	C
	CHSER (510), GA	T	T	T	T	R	R	A	W	W	C	T	C	C	C	C	A	T	R	T	C
Groups A/D	STMIN (101 AA), GA	Y	T	K	W	R	W	W	W	W	C	T	Y	C	C	C	A	T	~	~	~

~, base data not available

- **Little variation was noted in the ~600bp of the 18S rRNA gene region sequenced (did span V4 variable region). Phylogenetic analyses resulted in one large group with no divisions due to low bootstrap values.**
- **Based on several nucleotide differences, at least four groups were noted and these groups had representatives of basking and nonbasking species.**
- **Group A included (CHPIC, TRSCR, CHSER, STODO, DERET (chicken turtles, *Deirochelys reticularia*) and GRBAR (Barbour's Map turtle, *Graptemys barbouri*) from the United States and (BR, Black river turtles (*Rhinoclemmys funera*) and white-lipped mud turtles (MUD, *Kinosternon leucostomum*)) from Costa Rica.**
- **Group B included samples from PSCON, TRSCR, CHPIC, STMIN and a CHSER from the United States.**
- **Group C included a single sample of a snapping turtle (CHSER) from Canada (see Barta et al., 2013).**
- **Six turtles were co-infected with two genetic groups, five of which are likely infections with four having both groups A and B (n=4) and one with Groups A and D. A single mud turtle had a mixed infection with two unknown genetic types.**

CONCLUSIONS

- **Data from this study supports the hypothesis that turtle behavior (i.e., basking or non-basking) increases the prevalence and parasitemia of *Haemogregarina,* which is likely due to frequency of contact turtles have with leeches. However, there are within group parasitemias differences which indicates that either there are within group differences in leech exposure that may result in repeated re-infections (which hasn't been studied) or there are other factors that influence infection intensity.**
- **No difference in prevalence was noted between genders even though they have different intrinsic (e.g., testosterone in males) or extrinsic (e.g. females laying eggs on land away from leech vectors) factors that could impact infection dynamics.**
- **Based on DNA sequences, there is a possibility that different species of *Haemogregarina* are infecting the examined turtle species; however, the species are not host specific as each group contain different species of turtles.**
- **Additional data from different turtles in various locations are needed to better understand the ecology of this understudied group of parasites.**

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