

True Blood: Hematological comparison between wild and captive reptiles

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INTRODUCTION

Blood composition is crucial to proper immune function, energetics, and osmoregulation, among other processes. Microscopic examination of hematological parameters can provide information on an animal's current immune function (immature:mature erythrocytes and heterophil:lymphocyte ratios) and immune challenges (parasite loads); however, such studies are rarely undertaken in wild populations.

The ratio of immature to mature erythrocytes are often indicators of growth. Irregular levels can also correlate with compromised immune function due to energies directed toward parasite infection (Madsen et al. 2004). Innate immunity can be assessed by the ratio of heterophils:lymphocytes (H:L), and can also be used to indicate presence of a chronic infection (French et al. 2008). Parasite prevalence and stress may affect the distribution of these cell types.

Protozoans and other parasites often exist within reptiles through symbiotic relationships, but some relationships can be strictly parasitic and harm their host. High parasite prevalence can have immediate effects such as compromising the immune system and decreasing growth, and can also affect specific behaviors such as predator avoidance, foraging, and reproduction (Opplinger & Clobert 1997).

These parameters can be affected by captivity and urbanization. The hematological comparison of wild versus captive reptiles reported here is preliminary research for our study that will test the hypothesis that reptiles' immune function and parasite loads differ across an urban-rural gradient.

METHODS

Data Collection: We collected blood samples from six snake species (Fig. 1) at three field sites: Overton Park, Ames Plantation, and Lucius Burch Natural Area. We identified snake species and collected a 0.1ml blood sample from the caudal vein. We collected blood samples from four species of captive-bred lizards (Fig. 2) during tail removal for another study in the EPAC lab. We made blood smears and fixed slides on the day of collection. We stained slides using a modified Giesma stain protocol (Telford, 2009).



Figure 1. Wild species used in study: (A) Copperhead, (B) Ring-neck, (C) Rat, (D) Dekay's Brown, (E) Worm, and (F) Garter Snakes.

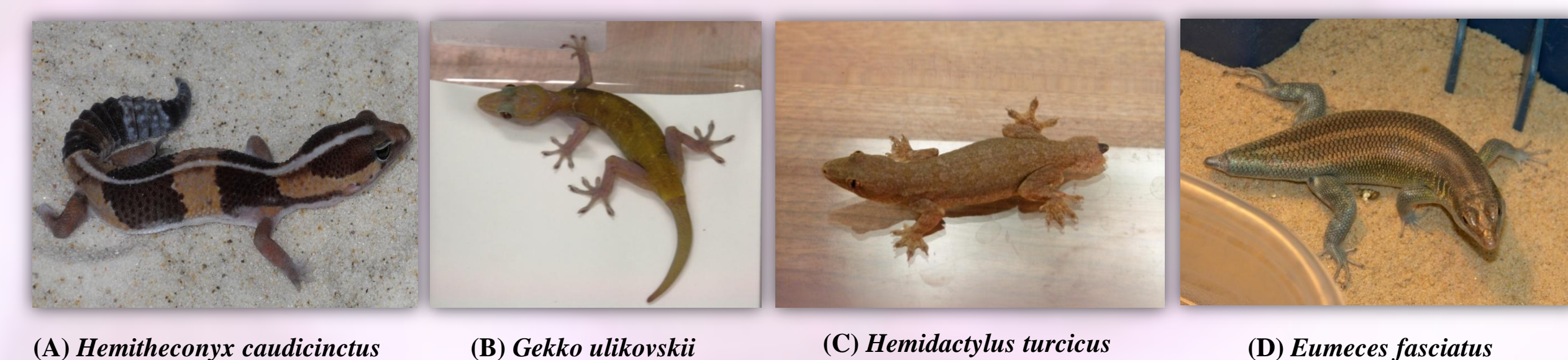


Figure 2. Captive species used in study: (A) African Fat-Tail, (B) Golden, and (C) House Geckos, and (D) Five-Lined Skink.

Data Analysis: We used an Olympus BX40 light microscope fitted with a Infinity digital camera to capture 25 random, non-overlapping images from each slide under 400x magnification. We analyzed slides using Adobe Photoshop CS4 to quantify: immature to mature erythrocytes ratios, H:L ratios, and intracellular parasite loads (Fig. 3). We used student's t-tests to compare these values in wild versus captive reptiles and used linear regression to determine whether the H:L ratio is correlated with parasite load of wild copperhead snakes.

RESULTS

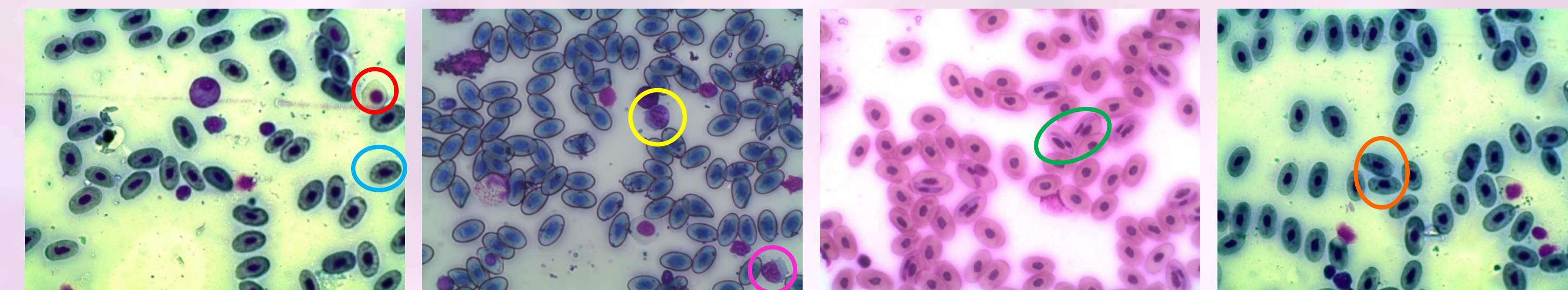


Figure 3. Hematological Parameters - Components quantified are circled: red=immature erythrocyte; blue=mature erythrocyte; yellow=heterophil; magenta=lymphocyte; green=haemogregarine infected erythrocytes; orange=plasmodium infected erythrocytes.

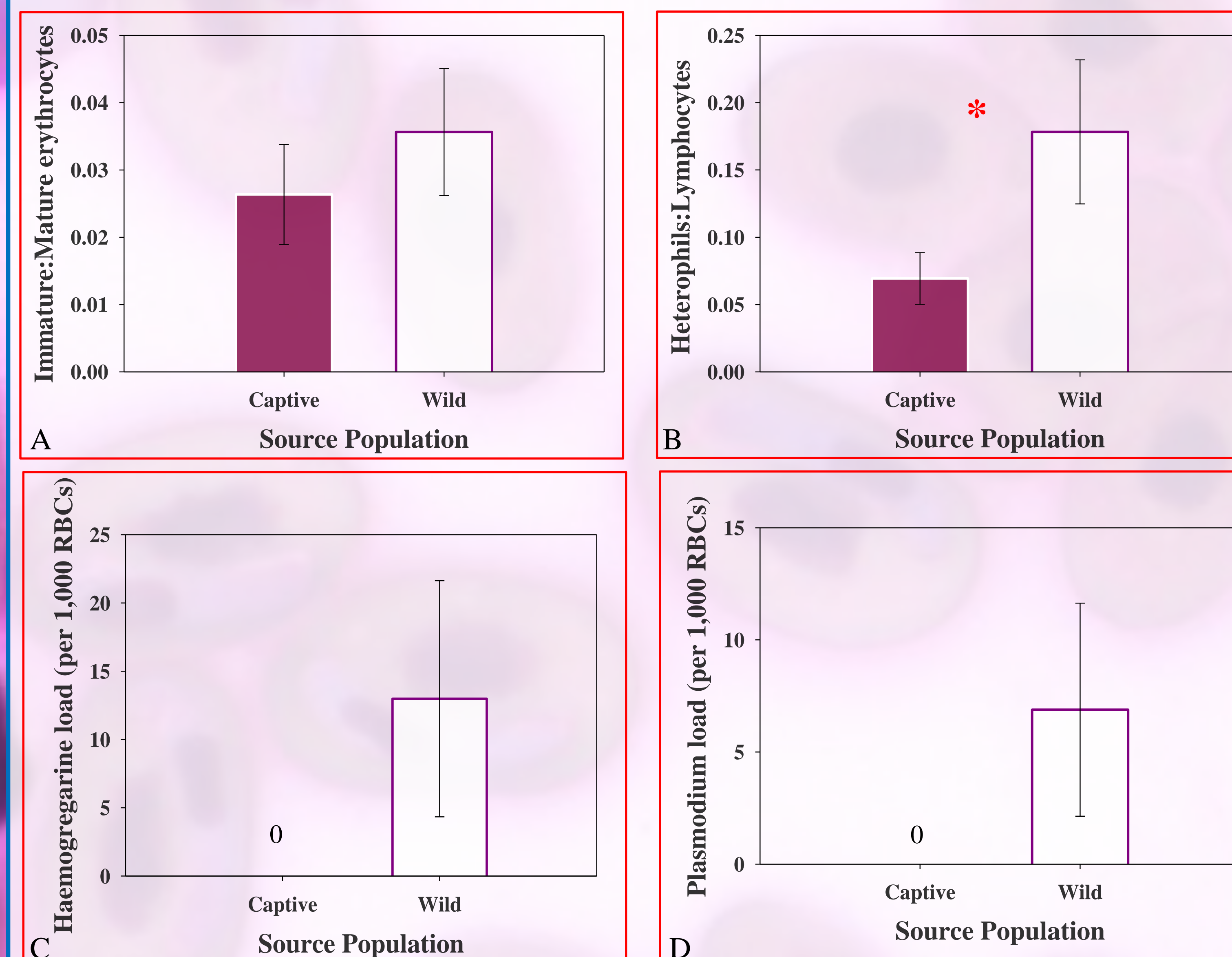


Figure 4. Hematological comparison of captive and wild species. (A) Immature:Mature erythrocyte ratio, (B) Heterophil:Lymphocyte ratio, (C) Haemogregarine load, and (D) Plasmodium load.

- Only Heterophil:Lymphocyte ratio was significantly different between captive and wild species
- Only wild snake species were infected with blood parasites
 - 60% of all wild snakes were infected – only copperheads
 - No infected animals contained both blood parasites – 83% Haemogregarine

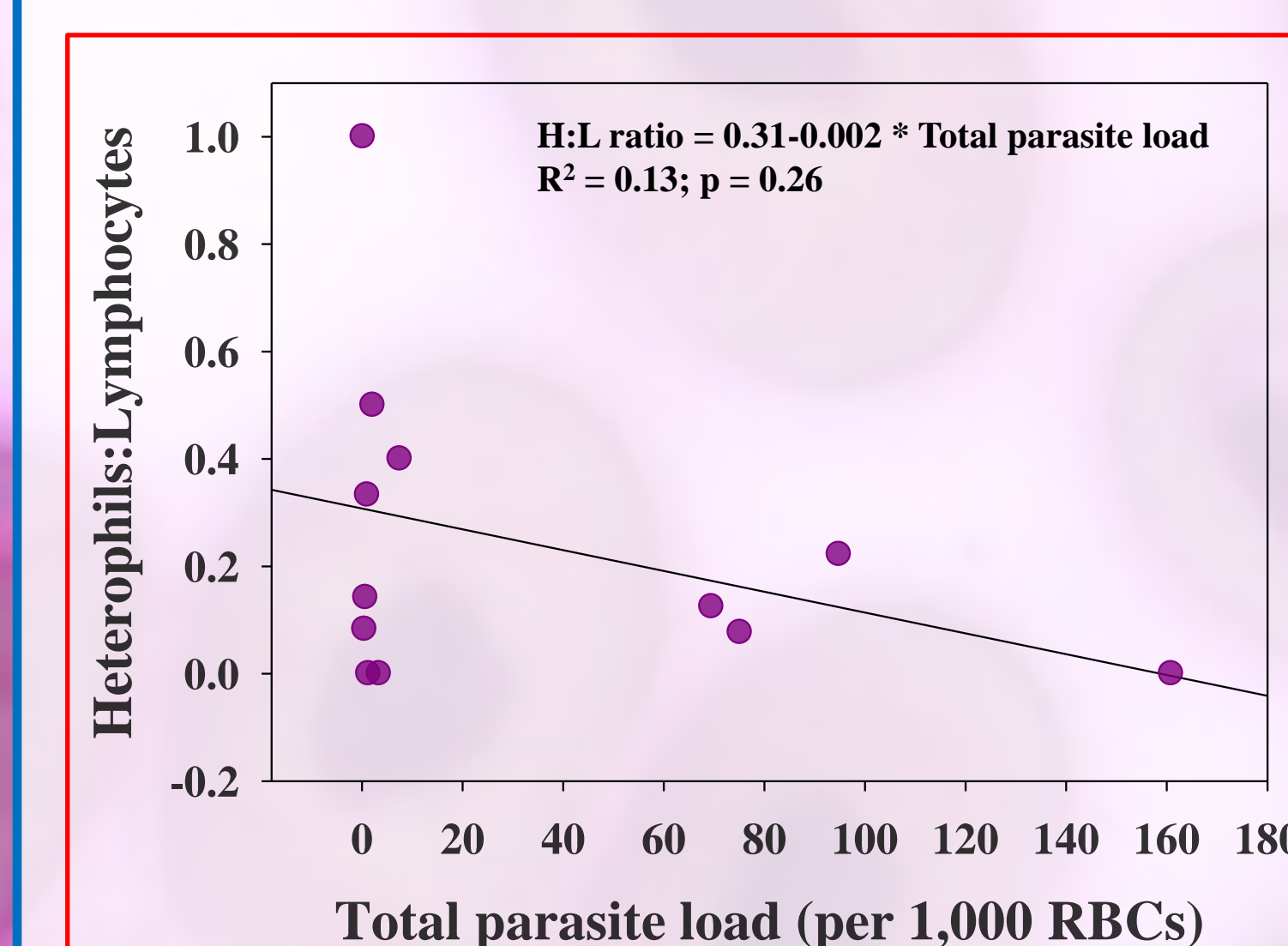
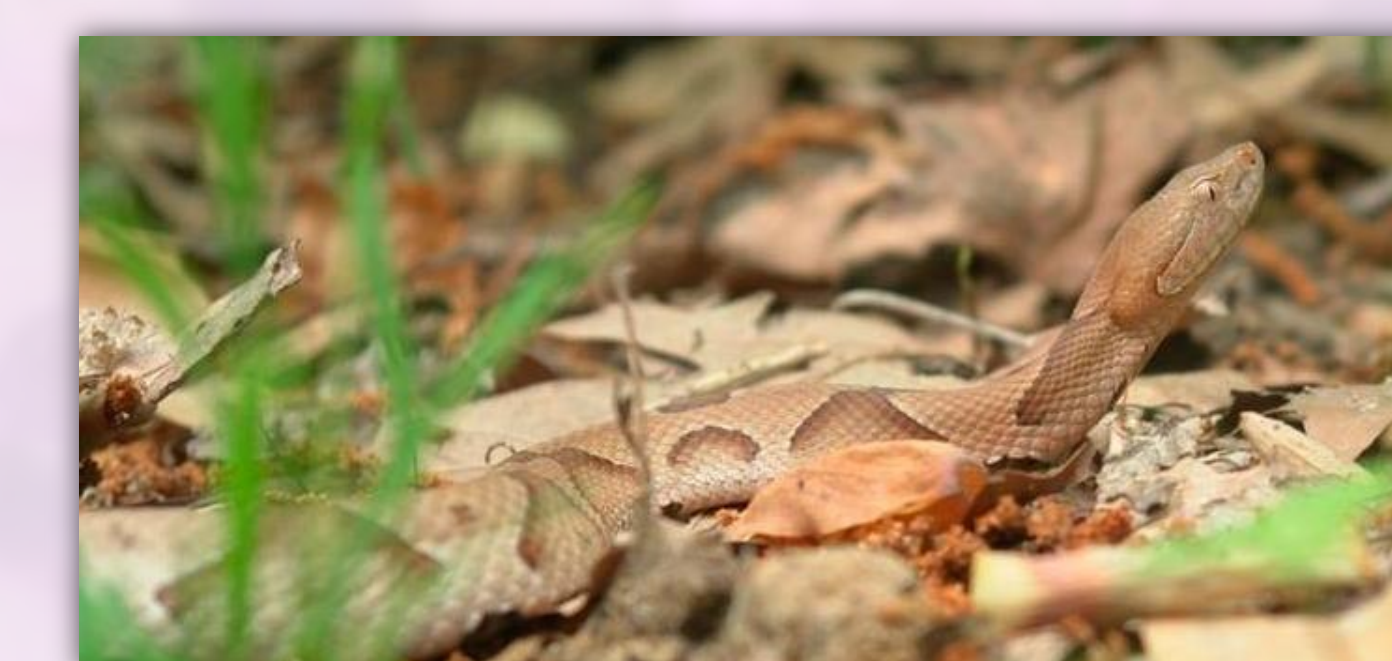


Figure 5. Relationship between H:L ratio and total parasite load in wild copperhead (*Agkistrodon contortrix*) snakes.



- Non significant negative correlation between H:L ratio and total parasite load

DISCUSSION

Immature:Mature erythrocyte ratio

• Similar immature:mature erythrocyte ratios suggest that captive and wild reptiles do not differ in growth rate (Fig. 4A).

Heterophil:Lymphocyte ratio

• Significantly greater H:L ratio in wild reptiles suggests they are fighting a chronic infection or are coping with an additional stressor (Fig. 4B).

➢ Captive-bred species were not infected by parasites (Fig. 4C&D) and have most likely become desensitized to some stressors.

➢ Urbanization introduces many novel stressors that may trigger an immune response in wild species.

Parasite Prevalence

• Only wild reptiles were infected with parasites, but parasite loads were low (Fig. 4C&D) and did not appear to surpass a threshold level that may affect blood composition.

➢ Lack of correlation between parasite load and H:L ratio in infected copperhead snakes suggests that parasite prevalence is not compromising wild species' immune systems (Fig.5).

➢ This lack of correlation suggests that the identified parasites are normally present in the species studied. Further study is needed to determine if high loads of these parasites affect blood composition or behavior.

FUTURE DIRECTIONS

•Geographic Information System (GIS)

➢ By increasing sample size and using GIS, we plan to determine if parasite loads vary across an urban-rural gradient.

•Polymerase Chain Reaction (PCR)

➢ PCR will allow us to conclusively determine parasite presence and identify parasite species.

•Intestinal Parasites

➢ Intestinal parasites are indicators of ingested parasites and will be an additional aspect of this study.

•Image Capture

➢ Increase magnification to 1000X to improve microscopic identification.

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