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Factors Affecting the Immune System of the Ornate Box Turtle (*Terrapene ornata*)

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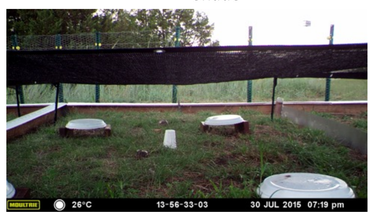
Introduction

The few studies of ectothermic immune function that have been conducted suggest that temperature plays a significant role in the modulation of immune activity[1]. Changes in temperature can elicit a stress response, which involves the secretion of glucocorticoid hormones. Corticosterone, the main glucocorticoid of the stress response, has been known to affect immune function, depending on the duration of the stressor. Typically, acute stress temporarily improves immune function, whereas chronic stress leads to immunosuppression[2]. Even less well-studied are the complex interactions between the stress response and immune function without corticosterone as the main focus. Though this study measured changes in corticosterone levels, it highlighted changes in immune function in the Ornate box turtle (*Terrapene ornata*) in relation to changes in body temperature and body mass due to a chronic stressor (high ambient temperature).

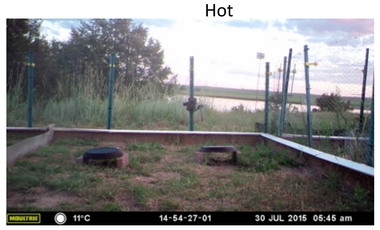
Materials and Methods

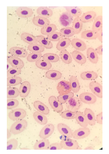
- Semi-natural enclosure housing 40 turtles, divided into two treatments: shade and hot.
- Blood samples collected from turtles at the start and end of the experiment.
- Microscope slides prepared and observed for white blood cell analysis (heterophil : lymphocyte ratio).
- Hemolysis-hemagglutination assays performed to measure level of antibodies[3].
- Experiment duration: July 7, 2015 to August 2, 2015.

Shade

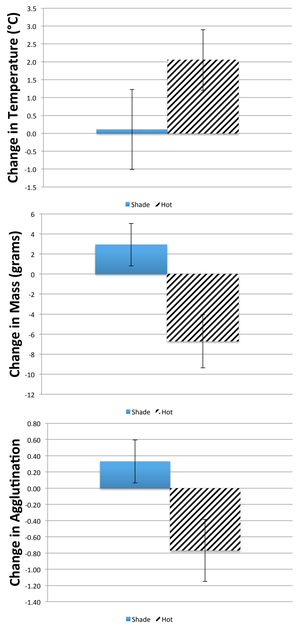


Hot



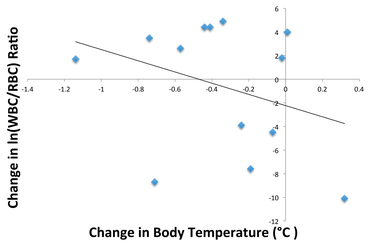


Results



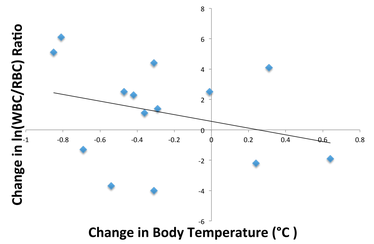
- Change in temperature was not significant across treatments (unpaired t-test: $p > 0.10$, $t = -1.667$, $df = 35.331$).
- Change in mass was significant across treatments (unpaired t-test: $p < 0.05$, $t = 2.836$, $df = 36.218$).
- Change in agglutination was significant across treatments (unpaired t-test: $p < 0.05$, $t = 2.366$, $df = 11.692$).

Shade treatment

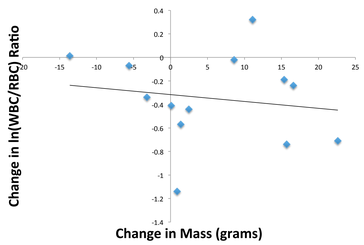


$Y = -4.7422x - 2.233$; $R^2 = 0.10741$

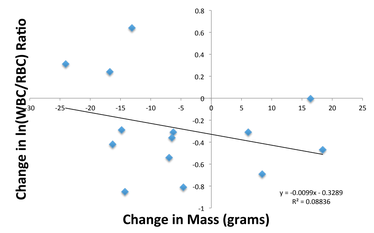
Hot Treatment



$Y = -2.2154x + 0.559$; $R^2 = 0.08506$



$Y = -0.0058x - 0.3164$; $R^2 = 0.02492$



$Y = -0.0099x - 0.329$; $R^2 = 0.08836$

- ΔT_b and Δmass had a significant effect on the ΔWBC/RBC ratio (general linear model: df model = 2, df error = 24, F ratio = 3.432, $p(\Delta T_b) = 0.0341$, $p(\Delta\text{mass}) = 0.0431$).
- No significant difference in the effects of ΔT_b and Δmass on ΔWBC/RBC ratio between treatments.

Conclusions

We propose that turtles exposed to heat stress, regardless of the treatment, may actually exhibit improved health due to observed reduction in immune activity. Specifically, turtles that gained body mass, had elevated body temperatures, and experienced a decrease in agglutination also correlated with a decreased WBC/RBC ratio from the beginning to the end of the study. Decreased agglutination and white blood cell counts in this case may have actually indicated improved body health. The increased body temperature served as an indicator that turtles were experiencing heat stress. In response to the chronic heat stress, turtles reduced their activity, staying buried as opposed to foraging, in order to minimize stress. This would explain the increased body mass. Without much activity, turtles in the hot treatment might have had less need for antibodies and circulating white blood cells. To eliminate undue risk of circulating white blood cells, turtles might have also decreased their white blood cell counts[1]. The decrease in the WBC/RBC ratio observed in correlation with decreased agglutination indicated lowered immune activity and, possibly, improved health in chronically heat stressed turtles.

Future Directions

Further studies are required to investigate the relationship between chronic heat stress and lowered immune activity as well as to clarify the complex interactions that take place between the reptilian stress response and immune function.

References

1. Zimmerman LM, Vogel LA, Bowden RM. Understanding the vertebrate immune system: insights from the reptilian perspective. The Journal of Experimental Biology 2009;213:661-671.
2. Dhabhar FS. Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection and immunopathology. Neuroimmunomodulation 2009;16(5):300-317.
3. Matson KD, Ricklefs RE, Klasing KC. A hemolysis-hemagglutination assay for characterizing constitutive innate humoral immunity in wild and domestic birds. Developmental and Comparative Immunology 2005;29:275-286.