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THE EFFECT OF *PLASMODIUM FLORIDENSE* ON RELATIVE LEUKOCYTE COUNTS OF *ANOLIS SAGREI* AND *A. CAROLINENSIS* IN FLORIDA, USA

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ABSTRACT: Native Green Anoles, *Anolis carolinensis*, and invasive Brown Anoles, *Anolis sagrei*, are commonly found in Florida and may be infected with the malarial parasite, *Plasmodium floridense*. Because no studies have directly addressed health effects of the parasite on Florida anoles, we collected blood smears of infected and uninfected anoles from Central and Southwest Florida and compared the overall leukocyte (WBC) counts, eosinophil counts, and heterophil/lymphocyte ratios. Eosinophils are generally elevated in response to protozoal infection and heterophil/lymphocyte ratios are often altered due to stress. A generalized linear model that tested contributions to erythrocyte/leukocyte ratios included infection status and locality as significant factors. We found significant differences in WBC counts between infected and uninfected lizards in Central Florida but not in Southwest Florida. Central Florida anoles also had higher mean WBC counts than Southwest Florida anoles. We did not detect significant differences in eosinophil counts or H/L ratios related to infection status. Our project is the first to examine leukocyte effects of *Plasmodium* infection in anoles and to provide leukocyte profiles of *Anolis* lizards. It appears that infected anoles sustain some negative immunological effects, at least in Central Florida. The differences in regions may be caused by the fact that Central Florida anoles still are under continuous interspecific competition whereas the Southwest Florida Brown Anoles are not because of low populations of Green Anoles. Additional studies that address leukocyte levels related to *Plasmodium* infection are needed to tease out the health and fitness effects on the lizards of Florida.

Key Words: anoles, *malaria*, *white blood cells*, *red blood cells*, *Dactyloidae*, *eosinophil*, *heterophil/lymphocyte ratio*, *protozoan.*

INTRODUCTION

In Florida, both *Anolis carolinensis,* the sole native anole species (Green Anoles), and *A. sagrei,* a successful invasive anole species (Brown Anoles), are susceptible to *Plasmodium floridense,* a species of malarial parasite that ranges through Middle America, the Caribbean, Florida, and Georgia (Garnham 1966, Schall, 1996, Telford 2009). Doan et al. (2019) found that *A. sagrei* had a lower infection prevalence than *A. carolinensis* in Central Florida, which may be caused either by a genetic or an ecological factor (Falk et al. 2011).

Plasmodium infection has detrimental effects in some species of lizards. For example, *Sceloporus occidentalis* produced smaller clutches when infected with *P. mexicanum* (Schall 1983) and infected males were less able to defend territories than uninfected lizards (Schall and Houle 1992). *Plasmodium* infection has also been shown to reduce male testis size, change male coloration, and decrease running stamina (Schall 1990). In some cases, *Plasmodium* infection in reptiles may cause severe anemia (Telford 1984) or premature death (Schall 1996). For lizards from the genus *Anolis* in Florida, there has not been sufficient research into the health effects of malarial parasites to determine their virulence or effects on fitness (Jordan and Friend 1971, Doan et al. 2019).

Although the exact mode of infection is not known, the anoles are most likely parasitized through the mosquito vector *Culex erraticus* feeding on their blood (Klein et al. 1988). *Culex erraticus* is a crepuscular feeder of a wide variety of vertebrate hosts (Cupp et al. 2004, Gray et al. 2011). Insect vectors inject *Plasmodium floridense* sporozoites (the parasite's larval stage) into lizards and the parasites enter the membrane of the red blood cell and develop into the meront life stage (Jordan and Friend 1971). The meronts then develop into gametocytes, which are the sexual life stage of the parasite that may be

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transmitted to the vectors to complete the parasite's life cycle (Garnham 1966, Jordan and Friend 1971). Unlike some other *Plasmodium* species, *P. floridense* specializes on erythrocytes (i. e. red blood cell = RBC) and does not infect leukocytes (WBC) (Schall 1996, Telford 2009).

If *Plasmodium* does cause health effects in Florida anoles, it is likely that an immunological response may be detected (Jenkins-Perez 2012). Sick animals may have reduced erythrocytes or altered leukocyte levels because of infection (Grasman et al. 2000, Bonadiman et al. 2010, McFarland et al. 2012). McFarland et al. (2012) found a 14% increase in total leukocyte counts in malaria-infected *S. occidentalis.* Doan et al. (2019) found that *Anolis* infected by *P. floridense* had lower red blood cell to white blood cell ratios in Central Florida, meaning that relative white blood cell counts were higher in infected lizards. We sought to expand upon that study to increase sample size, include a wider geographic range, and examine specific classes of WBCs.

Lizards have several types of white blood cells, including heterophils (which are homologous to neutrophils in mammals), eosinophils, lymphocytes, monocytes, basophils, and azurophils (which are unique to reptiles), each with different immune functions to combat different threats that may enter the bloodstream (Stacy et al. 2011, Jenkins-Perez 2012). Eosinophils are generally rare in squamates (Sypek and Borysenko 1988, Claver and Quaglia 2009), but are often elevated in response to parasitic infection such as protozoans or helminths (Stacy et al. 2011). Eosinophils are defenders against the early stages of parasite infection (Thrall et al. 2004). In humans, eosinophil levels change dramatically over the course of infection (Kurtzhals et al. 1998) and vary with severity of infection (Saidu et al. 2015). Motz et al. (2014) found no significant difference between *Sceloporus occidentalis* infected with *Plasmodium mexicanum* versus uninfected. To our knowledge, no studies have assessed the relationship of eosinophil levels and *Plasmodium* infection in anoles.

An additional way to assess health of animals involves measuring the heterophil/ lymphocyte ratios (H/L) (Davis et al. 2008). High levels of glucocorticoids (stress hormones) may cause increases in heterophils and decreases in lymphocytes (Thrall et al. 2004, Davis et al. 2008). Measuring H/L ratios as an indicator of stress has been done in a variety of vertebrates (Shutler et al. 2009, Das and Mahpatra 2014, Cotter 2015, Forget et al. 2017), but rarely in reptiles (Davis et al. 2008, McFarland et al. 2012). Only a single study has examined H/L ratios in relation to *Plasmodium* infection. McFarland et al. (2012) found that lymphocyte levels were increased but heterophils were unchanged in *S. occidentalis* lizards infected with *P. mexicanum.* In that study, H/L levels were lower in infected lizards (McFarland et al. 2012). No previous study has attempted to correlate H/L ratios to *Plasmodium* infection in anoles.

Geographic differences in immune function have not been studied in detail in lizards. Because parasite and vector presence and prevalence may vary geographically (Bennett et al. 1995, Sol et al. 2000), immunity may also differ by location (Dupas and Boscaro 1999, Barbosa et al. 2007). In other vertebrate and invertebrate species, immune function has been demonstrated to change clinally, change with temperature, or vary with other unstudied factors (Barbosa et al. 2007, Dionne et al. 2007). We studied two distinct regions of Florida to assess whether there were spatial differences in immune cell counts.

The overall objective of our project was to determine if *Plasmodium floridense* negatively affects the health of *Anolis carolinensis* and *A. sagrei.* We accomplished this through hematological examination with four related questions: (1) Does the ratio of RBCs to WBCs in *A. carolinensis* and *A. sagrei* infected by *P. floridense* differ from that of uninfected specimens? We hypothesized that the presence of *P. floridense* in *Anolis* would cause an elevation of WBCs relative to RBCs in infected lizards. (2) Do eosinophil levels differ between infected and uninfected lizards? Our second hypothesis predicted that there would be an increase in the number of eosinophils relative to other WBCs in the *Plasmodium*-infected lizards compared to the uninfected anoles. (3) Do heterophil/ lymphocyte ratios differ between infected and uninfected anoles? We predicted that we would find altered ratios in infected anoles compared to uninfected lizards, but we could not predict the direction because of conflicting previous studies. (4) Are there geographic differences in RBC/WBC ratios, eosinophil levels or H/L ratios? Because the Florida climates of Central and Southwest Florida do not differ greatly, we did not predict differences in these factors between the two localities.

MATERIALS AND METHODS

To determine leukocyte counts, we examined blood smears of *Anolis sagrei* from Sarasota County, Southwest Florida, collected in January 2018, and *A. sagrei* and *A. carolinensis* from Orange County and Seminole County, Central Florida, collected March through June 2016 during a previous study (Doan et al. 2019). Perkins et al. (2009) previously demonstrated that examination of blood smears and molecular screening yielded consistent results. The two regions of Florida are separated by approximately 240 km. We caught anoles opportunistically and during dedicated searches either by hand or with a slip noose from trees, buildings, the ground, or other structures. We recorded their snout-to-vent length (SVL) in mm with a digital caliper and sex class (male/female/juvenile). We collected a blood sample by clipping the third toe of the left hind foot with scissors. In Southwest but not in Central Florida, we used benzocaine (Orajel™) as a local anesthetic for the anoles by applying it to the toe to be clipped prior to measuring the SVL. The toe clip additionally served as a permanent mark and excluded marked lizards from recapture. We smeared blood from the toe clipping onto a microscope slide and then allowed it to air dry before fixing in a methanol fixative from Hema 3 Stat Fix Kit (Fisher HealthCare™).

We stained the blood samples using the Hema 3 Stat Fix Kit in the laboratory. After staining, we examined slides under 600x or 1000x total magnification. We determined the presence of *Plasmodium* parasites in the anoles through a 5-min scan of the slide (Doan et al. 2019). In a separate manual count to determine RBC/ WBC ratios, we counted both RBCs and WBCs using a cell counter until 100 WBCs were counted (Davis et al. 2008). We performed this count similarly to a differential leukocyte count with the blood cells counted around the edge of the blood smear (Doan et al. 2019). We separately recorded monocytes, heterophils, azurophils, eosinophils, basophils, and lymphocytes. We distinguished the characteristics of each WBC type using Strik et al. (2007) and Jenkins-Perez (2012).

To determine the factors contributing to RBC/WBC ratios of anoles, we performed a negative binomial generalized linear model (GLM) with a log link function on

blood cell counts. Factors included in the global model were infection status, locality, species, SVL, and sex class (male/female/juvenile). We included models that had each of the factors plus logical interactions (Table 1). We selected these factors because Doan et al. (2019) demonstrated that species and SVL may be important factors for determining infection prevalence and because sex class may be related to immunocompetence (Sheldon and Verhulst 1996). We tested 26 *a priori* candidate models and the best model was determined based on ΔAICc, considering models to have support when < 2 units from the highest ranked model (Burnham and Anderson 2002).

To test differences between the eosinophil counts of infected versus uninfected anoles, we used two sample Welch's *t*-tests for Central and Southwest Florida populations combined for *Anolis sagrei* and the single Central Florida region for *A. carolinensis*. We performed the same types of tests for H/L ratios. We similarly used Welch's *t*-tests to detect differences in eosinophil levels and H/L ratios between sites for *A. sagrei* with infected and uninfected lizards combined. All statistics were performed with IBM SPSS (version 23) and we considered results to be significant at $P < 0.05$. Means are presented ± SD.

RESULTS

We examined blood smears from 63 specimens of *Anolis sagrei* from Southwest Florida (6 infected/57 uninfected). The average RBC to WBC ratios were similar between infected (5.807 ± 1.79 RBC/WBC) and uninfected (5.72 ± 1.90) lizards (Table 2, 3). From Central Florida, we analyzed 47 blood smears (10 infected/37 uninfected) of *A. sagrei* and 23 (9 infected/14 uninfected) of *A. carolinensis*. For the uninfected *A. sagrei* the mean ratio was 4.82 ± 1.43 RBC/WBC and the infected *A. sagrei* ratio was 3.21 ± 1.53 RBC/WBC. For uninfected *A carolinensis* the RBC/WBC ratio was 4.68 ± 1.74 and for infected *A. carolinensis* it was 3.16 ± 1.11. Therefore, for both species in Central Florida, there appeared to be higher percentages of WBCs in lizards infected with *Plasmodium floridense*, but we did not see this result in Southwest Florida.

The candidate model with the highest AICc weight included only infection status and locality (Table 1). Other models with substantial support (i.e., ΔAICc < 2.0) included species and an interaction between infection status and locality (Table 1). The models that included SVL or sex were inferior with ΔAICc values >2.

Means of eosinophil counts at both sites combined were 16.94 ± 11.45 in infected *Anolis sagrei* and 18.24 ± 11.06 in uninfected, which was not a significant difference ($t = 0.424$, $P = 0.676$). Means of eosinophils for *A. carolinensis* in Central Florida were 15.33 ± 15.80 in infected and 20.64 ± 13.88 in uninfected, which was not a significant difference (Table 3; $t = 0.824$, $P = 0.422$). Mean percentages of eosinophils out of total WBCs in Central Florida were 12.4% for infected *A. sagrei,* 14.7% for uninfected *A. sagrei,* 15.3% for infected *A. carolinensis*, and 20.5% for uninfected *A. carolinensis*. In Southwest Florida eosinophil percentages were 20.3% for infected and 24.3% for uninfected *A. sagrei*. The difference in eosinophil percentages between sites was highly significant ($t = 3.426$, $P = 0.0009$).

Means of heterophil/lymphocyte ratios at both sites combined were 0.476 ± 0.350 in infected *Anolis sagrei* and 0.859 ± 1.835 in uninfected, which, though a seemingly large difference, was not statistically significant ($t = 1.838$, $P = 0.069$). Means of H/L ratios for *A. carolinensis* in Central Florida differed significantly (Table 2; $t = 2.244$, $P = 0.036$), with 0.243 ± 0.215 in infected and 0.418 ± 0.359 in uninfected. Means of heterophil/lymphocyte ratios of *A. sagrei* appeared be higher in Southwest Florida (0.909 ± 1.844) than Central Florida (0.661 ± 1.508), but the difference was not significant ($t = 0.774$, $P = 0.441$). The H/L ratios from all populations exhibited high variance (Table 2).

DISCUSSION

We used three hematological methods to assess the immunological consequences of infection by *Plasmodium floridense* on *Anolis sagrei* and *A. carolinensis,* finding that some effects could be detected. Our findings, however, were mixed and do not strongly support detrimental immunological responses caused by *Plasmodium* infection.

Table 1. Top 11 models from negative binomial generalized linear model AICc-based selection. The model explains the likelihood of red blood cell/white blood cell counts in *Anolis sagrei* and *A. carolinensis* individuals in Central and Southwest Florida, USA.

Model	df	Log Likelihood	AICc	ΔAICc	Akaike Weight
Infection, Locality	2	-937.329	1880.849	0	0.2832
Infection, Species	2	-937.932	1882.054	1.205	0.1550
Infection, Locality, Infection*Locality	3	-937.043	1882.407	1.558	0.1300
Infection, Locality, Species	3	-937.289	1882.900	2.049	0.1017
Infection, Locality, SVL	3	-937.322	1882.964	2.115	0.0984
Infection, Locality, SVL, Infection*Locality	4	-936.997	1884.478	3.629	0.0461
Infection, Locality, Species, Infection*Locality	4	-937.025	1884.534	3.685	0.0449
Infection, Locality, Species, Infection*Species	4	-937.206	1884.900	4.047	0.0374
Infection, Locality, Species, SVL	4	-937.277	1885.038	4.189	0.0349
Infection, Locality, SVL, Infection*SVL	4	-937.287	1885.058	4.209	0.0345
Infection, Locality, Sex	4	-937.305	1885.094	4.245	0.0339

Table 2. *Anolis sagrei* and *A. carolinensis* infected or uninfected by *Plasmodium floridense* in Central and Southwest Florida, USA, by sex class. Average red blood cell/white blood cell ratios (RBC/WBC) ± standard deviation by sex class and snout–vent length (SVL). Region refers to the region of Florida.

Species	Region	Infection Status	Sex Class	Sample Size (n)	SVL range (mm)	RBC/WBC
A. sagrei	Southwest	Uninfected	Male	37	32.8–64.2	5.96 ± 1.87
			Female	8	27.8–41.1	4.44 ± 1.96
			Juvenile	12	21.0–39.9	5.86 ± 1.79
A. sagrei	Southwest	Infected	Male	6	35.1–68.4	5.81 ± 1.79
			Female	0		
			Juvenile	0		
A. sagrei	Central	Uninfected	Male	24	36.5–48.2	4.68 ± 1.30
			Female	10	40.6–65.4	5.26 ± 1.88
			Juvenile	3	31.3–40.1	4.47 ± 0.49
A. sagrei	Central	Infected	Male	6	53.5–62.4	2.84 ± 1.54
			Female	4	42.1–48.9	3.22 ± 1.59
			Juvenile	1	38.8	5.05
A. carolinensis	Central	Uninfected	Male	7	42.5–59.4	5.02 ± 2.28
			Female	5	40.4–51.6	4.18 ± 1.20
			Juvenile	1	39.1	4.50
A. carolinensis	Central	Infected	Male	4	49.5–52.4	2.70 ± 1.26
			Female	5	40.4–46.8	3.52 ± 0.96
			Juvenile	0		

Differences in RBC/WBC ratios found in anoles from Central and Southwest Florida were determined by infection status and locality. According to our analysis, species and lizard body size may also be important in determining RBC/WBC ratios. *Plasmodium*-infected Central Florida lizards had higher relative levels of WBCs in their blood, which matches our expectations and the results from Doan et al. (2019), but there was no detectable difference in RBC/WBC ratios in the Southwest Florida infected versus uninfected lizards. Although we did not directly test the factors causing differences in RBC/WBC ratios, our results provide evidence that *P. floridense* may be related to an immunological response upon infection, at least in one region of its Florida range. Although some *Plasmodium* species invade white blood cells (Schall 1996), which could distort the relative blood counts, *P. floridense* does not infect WBCs (Thompson and Huff 1944) and therefore the differing levels of WBCs cannot be caused by that factor. Other than the preliminary tests by Doan et al. (2019), our study presents the first research that examined relative leukocyte levels in relation to *Plasmodium* infection in anoles.

We had predicted that eosinophils would be elevated in infected anoles because they are responsible for protection against protozoal parasites (Strik et al. 2007). Similar to Motz et al. (2014), we found no such effect, which may mean that eosinophils are not as important in protozoal immunological responses in reptiles as previously assumed. Alternatively, *Plasmodium* may not elicit an eosinophil response even if other protozoans do, or the low parasitemia of *P. floridense* in Florida anoles (Perkins et al. 2009, Doan et al. 2019) may not be enough to cause a detectable eosinophil response. In fact, the percentages of eosinophils in our lizards were similar to the 7–20% range reported by Frye (1991) for healthy reptile species. Although eosinophil levels change greatly throughout the year in temperate animals that exhibit torpor in the winter (Frye 1991, Strik et al. 2007), no such effect is expected at our subtropical localities because lizards are active throughout the year, though with variable activity levels (Losos 2009).

We also predicted that H/L ratios would be elevated in infected lizards. There was no significant difference in the H/L ratios of infected and uninfected lizards, although we found somewhat higher ratios in uninfected lizards than infected lizards in all populations (Table 2). We detected high variability in heterophil and lymphocyte counts, which has been demonstrated in previous reptile studies (Sypek and Borysenko 1988, Frye 1991). In general, we detected lower numbers of lymphocytes as proportions of total leukocytes in both infected and uninfected lizards than other published studies on reptile species (Table 3; Sypek and Borysenko 1988, Frye 1991, Thrall et al. 2004, Davis et al. 2008). As no leuko-

Table 3. Average leukocyte statistics for *Anolis sagrei* and *A. carolinensis* infected or uninfected by *Plasmodium floridense* in Central and Southwest Florida, USA, with all sex classes combined. Region refers to the region of Florida. All numbers are average percentages ± standard deviation except for RBC/WBC and H/L. RBC/WBC refers to red blood cell/white blood cell ratios; H/L is heterophil/lymphocyte ratios.

Species	Region	Infection Status	RBC/ WBC	Basophils	Eosinophils	Heterophils	Lymphocytes	Monocytes	Azurophils	H/L
A. sagrei	Southwest	Uninfected	5.72 ± 1.90	36.14 ± 13.0	20.52 ± 11.8	10.56 ± 7.7	18.45 ± 8.3	7.92 ± 4.2	7.40 ± 8.8	0.967 ± 1.9
A. sagrei	Southwest	Infected	5.81 ± 1.79	28.66 ± 5.8	24.50 ± 14.5	7.83 ± 5.4	21.83 ± 8.8	10.00 ± 3.4	8.66 ± 9.5	0.361 ± 0.4
A. sagrei	Central	Uninfected	4.82 ± 1.43	33.32 ± 11.3	14.72 ± 8.9	8.75 ± 6.8	26.32 ± 12.8	7.86 ± 4.9	9.56 ± 10.6	0.634 ± 1.5
A. sagrei	Central	Infected	3.21 ± 1.53	36.70 ± 9.7	12.40 ± 6.3	9.90 ± 5.7	23.80 ± 10.2	9.80 ± 5.4	7.40 ± 8.6	0.544 ± 0.4
A. carolinensis	Central	Uninfected	4.68 ± 1.73	32.71 ± 9.7	20.64 ± 13.9	9.72 ± 4.8	23.28 ± 8.6	10.5 ± 6.0	3.57 ± 2.9	0.413 ± 0.4
A. carolinensis	Central	Infected	3.16 ± 1.11	34.77 ± 12.0	15.33 ± 15.8	5.88 ± 4.0	28.33 ± 8.4	8.22 ± 4.7	8.33 ± 7.2	0.243 ± 0.2

cyte studies have been performed on *Anolis* lizards, we are the first to report leukocyte profiles of these species (Table 3). We suggest additional research with greater sample sizes that would have the power to demonstrate if there is a H/L stress response in *Plasmodium*-infected anoles of both species.

Although species was an important factor in second-ranked RBC/WBC model, leukocyte counts between the two species were similar in the sympatric areas of Central Florida. Both uninfected and infected lizards of both *Anolis* species from Central Florida had much lower RBC to WBC ratios (i.e. higher levels of WBCs) than their Southwest Florida conspecifics. Locality was the strongest factor in our model and the differences between regions of Florida were more substantial than the difference between the two *Anolis* species. Likewise, eosinophil counts between the two regions were also highly significant with Southwest Florida having higher eosinophil counts. These results were not expected, and an explanation why Central Florida anoles would have much higher numbers of WBCs and lower eosinophil percentages is not obvious. Other studies have also demonstrated geographic variation in leukocyte counts in birds, but did not offer explanations as to the causes (Grasman et al. 2000).

One possibility for this phenomenon could be the recency with which *A. sagrei* was introduced to those areas of Florida from their native Caribbean islands. *Anolis sagrei* were first introduced to the Florida Keys in the 1880s (Krysko et al. 2016), quickly spreading to major Florida seaports through continued accidental and intentional introductions from Cuba, the Bahamas, and other Caribbean islands (Oliver 1950). The first records of *A. sagrei* in Southwest Florida were in 1977 to Lido Key and Longboat Key in Sarasota County (Godley et al. 1981), though the species was abundant in the Tampa area, approximately 69 km north of Sarasota, by 1947 (Oliver 1950). In Central Florida, *A. sagrei* was first recorded in Orlando, Orange County, in 1978 (Godley et al. 1981). Although it is likely that anoles existed in both areas prior to those dates, these data show a miniscule variation in the years of introduction to the different collection sites. It appears that *A. sagrei* arrived in the areas nearly simultaneously, which would not explain why lizards of one region have much higher levels of WBCs than the other and why one region would demonstrate immunological effects of *P. floridense* infection whereas the other did not have differences in infected versus uninfected lizards.

Another important factor may be that *A. carolinensis* were still relatively common in our Central Florida sites, whereas they were very uncommon in our Southwest Florida sites (so uncommon that we did not include them in this study). If the two species of anoles are still in continuous competition in some areas but not others, their immune systems may be more stressed (Tian et al. 2015), causing higher levels of WBCs in such areas. It is possible that the continued competition between the two species in Central Florida creates an immunological stress response that is absent in Southwest Florida, where interspecific competition among anoles is virtually nonexistent. Our H/L locality results do not support the notion that Central Florida anoles are experiencing more stress than the Southwest Florida anoles because the Southwest Florida anoles had slightly higher H/L ratios. Thus, our results appear to point to a larger overall WBC increase in Central Florida but higher stress in Southwest Florida. The reasons for these opposing effects remain a mystery worthy of additional research.

Lizard body length was not a significant factor in the top GLM models, but did contribute to the fifth-ranked model (ΔAICc = 2.115). On average, infected *Anolis sagrei* were larger than uninfected lizards, but there were no size differences in *A. carolinensis*. Because producing and maintaining leukocytes in costly for animals (Nunn 2002), growing to a greater size is more difficult when animals are immunologically challenged such as with parasitic infection. Schall (1996) demonstrated that for some *Plasmodium* species infection was positively correlated with lizard size. Larger animals have more opportunity to become exposed to infectious agents because of their need to forage more (Schneeberger et al. 2013) or because their bodies have more surface area for attack by insect vectors (Port et al. 1980). In addition, larger lizards are older and have had more time to become infected with *Plasmodium,* which may be a chronic infection in Florida *Anolis* species (Schall 1996, Perkins et al. 2009). Larger lizards may be under more stress than smaller ones because they have to be engaged in reproductive activities or territorial defense that smaller lizards do not (Schall 1996). The relationship between anole size, leukocyte counts, and infection by *P. floridense* is an area of research in need of further study.

Our project is the first to examine leukocyte effects of *Plasmodium* infection and to provide leukocyte profiles of *Anolis* lizards. This avenue of research is promising because the slides previously created to examine infection could be used further to attempt to detect immunological responses associated with infection. Thousands of slides from a wide variety of reptiles and many species of *Plasmodium* are available for examination and could be the key to further elucidating leukocyte trends associated with *Plasmodium* infection from around the world.

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