

**Geographic Variation in the Lower Temperature Tolerance in the Invasive  
Brown Anole, *Anolis sagrei* and the Native Green Anole, *Anolis carolinensis*  
(Sauria: Polychrotidae)**

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*This thesis is dedicated to my parents, Efraín Rubio and Vivian Rocha, for their continual and comprehensive support throughout my life.*

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## Abstract

Invasive species are considered to be the second greatest threat to native biodiversity and several factors have been identified as contributing to the success of introduced species, including their initial genetic variation and the ability of populations to adapt to a new environment.

Temperature has a significant impact on reptilian ecology and distribution since they ordinarily rely on external heat sources for the maintenance of body temperatures suitable for normal activity. Body temperature affects performance in these organisms given its importance for all aspects of behavior, locomotion, courtship and rates of feeding and growth. Critical thermal tolerances can, therefore, give an indication of the range of climatic conditions that can be tolerated and which may be the causal range limit in some cases. We studied the cold tolerance (Critical Thermal Minima) of female and male invasive *Anolis sagrei* and native *Anolis carolinensis* (Sauria: Polychrotidae) in four populations along a latitudinal gradient from south Florida to northern Georgia. Cold tolerance (CTMin) was measured under field conditions and after a period of acclimation to identify whether there is variation in this characteristic as latitude increases reflecting differential selection for lower temperature adaptation or phenotypic plasticity and whether there is a difference in cold tolerance between the native and invasive species. We found a geographic cline in field CTMin for both species; the lowest CTMins were exhibited by those anoles from the northern-most population (Savannah, GA). In all four populations *A. carolinensis* has a lower field cold CTMin than *A. sagrei*, and for both species, male CTMin is lower than female CTMin. However, after a period of acclimation, there were no significant differences in CTMin among populations or between sexes of either species, suggesting a notable capacity of both species to acclimate to local conditions.

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## Introduction

Invasive species are considered to be the second greatest threat to native biodiversity following habitat destruction (Wilson 1992, Vitousek *et al.* 1997). Introduced species are those that have moved beyond their native range aided by human activities, have established in a new area, proliferate, spread and persist in their new range to the detriment of the environment. Several factors have been identified as contributing to the success of introduced species in general, including those which come to be considered invasive. In the area into which they have been introduced, they may find better physical conditions, more abundant resources, and empty niches relative to their native ranges. In addition, they may be released from predators or competitors, parasites or pathogens. These differences may allow newcomers to escape the biotic constraints that regulate their populations in their native land (Mack *et al.* 2000).

The brown anole, *Anolis sagrei* Duméril & Briçon (Polychrotidae), is remarkable for its colonizing ability, which is reflected in its wide geographical distribution. The species is thought to have evolved in Cuba from which it colonized the Bahamas, Little Cayman, Cayman Brac, Swan Island, and the Atlantic versant of Central America (Williams 1969). There is controversy as to whether the population on Jamaica is native or was facilitated by early human inhabitants of the Caribbean region. The origin of the population in lower Florida keys, first reported by Garman (1887) in Lee (1987), is likewise uncertain.

*Anolis sagrei* appears to have arrived on the mainland of South Florida by 1940 (Lee 1985), as the result of introductions from both Cuba and the Bahamas, which subsequently interbred (Kolbe *et al.* 2004). It has exhibited an exponential range expansion since its introduction and now occurs throughout Florida and into Georgia, Louisiana and Texas

(Campbell 1996). Recently, apparently established populations have been documented in South Carolina (Turnbough 2006) and North Carolina (Beane & Corey 2010), *Anolis sagrei* is considered highly invasive, reaching densities of 1.2 individuals per m<sup>2</sup> and negatively impacts populations of the native green anole, *Anolis carolinensis* (Campbell & Echternacht 2003).

Until the introduction of *A. sagrei*, *A. carolinensis* was the only representative of the genus inhabiting North America. The geographic range of *A. carolinensis* includes the southeastern United States from the east coast to Oklahoma, Arkansas and Texas and as far north as Tennessee and North Carolina (Conant & Collins 1998). This distribution represents the northern limit of the range of the entire genus. Williams (1969) suggested that its range is limited in the north by temperature and in the west by moisture. There have been suggestions that the *Anolis sagrei* might negatively affect the *A. carolinensis* in Florida via interference competition (Collete 1961) and the exclusion of *A. carolinensis* by *A. sagrei* in coastal areas of south Florida, Mexico and Belize has been attributed mainly to the lack of preferred habitats of *A. carolinensis* and competitive exclusion by *A. sagrei* (Williams 1969, Crews 1980). This could be a rapid process since Christman (1980) and Campbell (2000) noticed that green anoles became rare or were absent at sites in Florida within a few years of the arrival of *A. sagrei*.

Trying to understand the factors that affect the distribution of species on earth has been one of the most studied topics in ecology, and although we know that biotic interactions and climate play a major role determining distributions of organisms, the exact way in which climate, particularly temperature, constrains geographical distributions is still not well understood. This topic is especially of interest when considering invasive species, since the extent of the range expansion greatly depends on the conditions that can or will be tolerated (Root 1988, Crozier 2003). Temperature has a significant impact on reptilian ecology and distribution (Cowles &

Bogert, 1944), since they are ectotherms and ordinarily rely on external heat sources for the maintenance of body temperatures suitable for normal activity. Body temperature is considered the most important ecophysiological variable affecting performance in these organisms, given its importance for all aspects of behavior, locomotion, immune functions, sensory input, foraging ability, courtship and rates of feeding and growth (Angilleta *et al.* 2002). Thus, physiology and more specifically, critical thermal tolerances can give an indication of the range of climatic conditions that can be tolerated and may be the causal range limit in some cases (McConnachie *et al.* 2007). Critical thermal minimum is known to be the minimum temperature at which individuals performance/locomotion is possible, and also corresponds to the “ecologically lethal” temperature, since individuals will not be able to escape further decreases in temperature that will lead eventually to their death (Cowles & Bogert, 1944, Doughty 1994). So, as latitude increases and individuals experience an increase in the intensity and duration of the winter, lower mean annual temperatures and lower extreme temperatures, they have to be able to confront them directly through immediate or evolved modifications in the physiological response that allow them to have higher resistance to cold temperatures.

Anoles do not hibernate. During the winter in East Tennessee, *A. carolinensis* seeks shelter in retreats in exposed rock crevices or holes in the ground. They emerge and are active on any day that the substrate is exposed to the sun and becomes sufficiently warm (Bishop & Echternacht 2004). When they are active during the winter, the body temperatures of anoles approximates those recorded during the summer but they must not venture so far from their retreats that they are exposed to temperature reductions that prevent their return to the retreats before they become immobile and risk death. Therefore, tolerance to relatively low temperatures may be adaptive for populations of *A. carolinensis* at the northern extreme of its range where it is

exposed to a highly seasonal environment where winter temperatures often approach or exceed freezing. Wilson and Echternacht (1987) demonstrated that the critical thermal minimum temperature (CTMin) of a population of *A. carolinensis* in East Tennessee, at the northern extreme of the range of the species, was significantly lower than of populations in Central Florida and southern Georgia. The difference is suspected to have a genetic basis. Denesha (2006) explored the thermal tolerance of three populations of *A. sagrei*, two from the southern (Miami and Naples) and one from the northern (Lake City) part of Florida. He found some evidence that northern populations of *A. sagrei* had greater tolerance to cold temperatures than southern populations and that the temperature of acclimation could influence the CTMin of the populations. However, the methods used by Denesha to determine CTMin did not allow him to find the greatest degree of cold acclimatability, so his results only suggests that the thermal sensitivity has changed in northern populations.

Fossil *A. carolinensis* are known from cave deposits in northwestern Georgia and northeastern Alabama that date from ca. 10,000 and 26,500 years before present, respectively (Holman 1995). Neither the colonization history of *A. carolinensis* nor its rate of northward movement following colonization is known and, therefore, neither is the rate at which adaptation to low temperatures occurred. *Anolis sagrei*, on the other hand, began its northward range expansion about 70 years ago and its rate of progress is well-documented (T. C. Campbell, pers. comm.). Therefore, it is of interest to identify the lower thermal tolerance in *A. sagrei* as it follows the path of *A. carolinensis* northward and whether there is geographic variation in this trait as a result of phenotypic plasticity.

In this study I examine if there is geographic variation in the lower thermal tolerance in *A. sagrei* and whether the pattern is similar to that of *A. carolinensis* along the same latitudinal

gradient. It is expected that at higher latitudes individuals of both species will have higher cold tolerance (lower CTMin, reflecting differential selection for lower temperature adaptation or phenotypic plasticity) and since *A. sagrei* has only recently begun its range expansion, it is expected to have a lower tolerance to cold temperatures than the native green anole in all four populations. I also compare cold tolerance between sexes of both *A. sagrei* and *A. carolinensis* (only males were studied by Wilson and Echternacht 1987); since it is known that genders of lizards can differ conspicuously in ecology and behavior (Schoener 1967, Spoecker 1967, Stamps *et al.* 1997, Butler *et al.* 2000), possibly encountering different operative thermal environments (Huey & Pianka 2007) that could affect differently their response to cold temperatures.

## Materials and Methods

### *Field Study-*

Between June 22<sup>nd</sup> and July 31<sup>st</sup>, 2011 anoles were sampled at five localities along a latitudinal transect from the vicinity of Savannah, Georgia to Naples, Florida (Fig. 1). At each site, between 0900 and 1800, a minimum of nine adult males and nine adult females of each species (except as noted) were captured by noose. Only *Anolis sagrei* were collected at the Naples site because few *A. carolinensis* were found there, possibly because of the abundance of *A. sagrei* and another introduced saurophagic anole, *A. equestris*. Only *A. carolinensis* were collected at the Punta Gorda site. Anoles from Naples and Punta Gorda sites were treated as the southernmost population for both species in the analyses, and treated as belonging to “South, FL” hereafter. Climatic data were five year averages (2007-2011) for the month of January, the coldest month of the year for all the populations under study, and other climatic data were obtained from the National Climate Data Center (Table 1).

Field variables were measured immediately after capture of each individual to evaluate the relationship of their microhabitat, size and mass with cold tolerance. Body temperature and air temperature 1cm and 1m above the perch were measured to 0.2 °C using a Miller & Weber cloacal thermometer. Perch height and perch diameter were measured to the nearest mm using a 2m foldable ruler, and vegetation cover was measured using a Forestry Suppliers spherical densitometer. Snout-vent length (SVL) was measured to the nearest mm using a clear plastic ruler, and weight was determined to the nearest 0.1g using a 10g Pesola© spring scale.

Field Critical Thermal Minimum (FCTMin) was determined for each lizard following the methods of Spellerberg (1973) to identify the cold tolerance of the individuals acclimatized

to their particular environments and to determine an appropriate acclimation temperature for subsequent assessment of CTMin in the laboratory (LCTMin). For determination of FCTMin, the acclimatized lizards were placed on a ca. 3 mm thick piece of acrylic plastic placed on top of the ice in an ice-filled plastic cooler. They were cooled until they reached their CTMin, the temperature at which they were unable, after 30 seconds, to walk after being stimulated in the hind legs and dorsal region with a fine brush and would, therefore, be unable to escape further reductions in temperature. I used the temperature at which the lizards stop walking instead of the righting response as the endpoint because individuals of *A. sagrei* would become immobile when put on their back before the cooling would begin, at ambient temperature of approximate 25°C. Lizard body temperature was monitored using an OMEGA© HH508 thermoelectric potentiometer with a resolution of 0.1°C, and a 30 gauge copper-constantan thermocouple inserted 0.5 cm into the cloaca and taped with surgical tape to the base of the tail. All field tests were conducted in the evening of capture between 1800 and 1200 in order to account for potential circadian rhythm effects (Spellerberg & Hoffman 1972).

#### *Laboratory Study-*

Physiological acclimatization over periods of time, ranging from minutes to seasons, can shift the FCTMin of an ectothermic animal. Thus, at temperate latitudes, for example, seasonal differences in FCTMin may be observed. There are, however, limits in the extent to which CTMin can be shifted through acclimatization. The FCTMin may not represent the absolute limit of tolerance to low temperature (i.e., the “incipient” or “ultimate” limits of Spellerberg 1973) which has resulted from adaptation that has occurred over multiple generations. In this study, mean FCTMin for male and female lizards from each population were compared in common garden fashion to mean LCTMin values obtained following acclimation to

the same ambient temperature. Lizards captured in the field were brought to the University of Tennessee, and housed individually in 3.78 l screen-topped clear glass jars provided with a wooden perch and water dish. They were fed crickets dusted with a vitamin D supplement on alternate days and water was provided *ad libitum* over a 69 day period at 25°C +/- 2°C with a photoperiod of 12L:12D. Immediately prior to testing, each lizard was subjected to a 48 hr period of acclimation at a temperature of 10°C, 1°C below mean FCTMin values, the acclimation protocol which has been found to provide the greatest degree of acclimatability (Spellerberg 1973). Lizards were subjected to fasting 48 hours prior to and during the period of acclimation to account for possible effects of feeding status on cold tolerance; water was always provided. LCTMin was determined following methods identical to those in the field. After the test, each lizard was returned to an environment at 25°C and food and water was offered.

#### *Statistical analyses-*

All data were tested for normality (Kolmogorov-Smirnov test) and homogeneity of variances (Levene's test). T tests and one way ANOVAs were used to determine statistical differences in mass between sexes and between populations of *A. carolinensis* and *A. sagrei*, ANOVAs were followed by a Tukey's Honest Significant Difference test. Pearson correlation tests were used to determine the relationship between FCTMin and field variables: mass, body temperature at capture, SVL, perch height, perch diameter and vegetation cover of female and male *A. carolinensis* and *A. sagrei*. Because mass was found to be significantly related to FCTMin, an analysis of covariance (ANCOVA) was used to test for geographic variation in FCTMin, with FCTMin as the dependent variable, population, species and sex as effects, and mass as the covariate. The interaction effects between species, sex and population in the FCTMin were analyzed using a Full Factorial ANOVA.

Daily Minimum and Maximum temperature for each month of the year between 2007-2011 were compared between field sites using a one-way ANOVA followed by Tukey's Honest Significant Difference test ( $P < 0.05$ ). Pearson's correlations were used to identify relations between Monthly Mean Minimum Temperature (MMNT), Extreme Minimum Temperature for the month (EMNT), number of days with minimum temperature equal or below 0°C (DT0°) with FCTMin and Body Condition Index (BCI); calculated as the residual score from Ordinary Least Squares (OLS) general linear regression of natural log transformed mass against natural log transformed SVL (Schulte-Hostedde, *et al.* 2005). The BCI of each individual, measured as the weight (mass) relative to the length (SVL) was compared between sex of each species using an ANCOVA with mass as the dependent variable, sex as effect and SVL as covariate (García-Berthou 2001). Lizards that weigh more than predicted by SVL are regarded as being in relatively good condition (Weatherhead & Brown, 1996).

Because laboratory sample sizes from the two southernmost populations (Naples and Punta Gorda, FL) were small, these sites were excluded from the analyses of LCTMin. Pearson correlations were used to identify relations between field variables, including FCTMin, and LCTMin. A repeated measures ANOVA with within subjects test was used to test for the effect of acclimation on the individuals. LCTMin was compared between sexes, species and populations with a Full Factorial ANOVA. Differences were considered to be statistically significant when  $P < 0.05$ .

## Results

### *Field Study-*

Male *A. carolinensis* ranged in SVL from 44mm to 66mm (n=52); females ranged from 41mm to 53mm (n=57) (Table 2). Male *A. sagrei* ranged in SVL from 42mm to 62mm (n=46); females ranged from 38mm to 48mm (n=48) (Table 2). There was a latitudinal gradient in the SVL of the largest male collected at each site for *A. carolinensis*, ranging from 57mm in south Florida to 66mm at Savannah, GA. No latitudinal trend was noted for females of either species or males of *A. sagrei*. There was no significant correlation between SVL and FCTMin for either sex of either species (Male *A. carolinensis*:  $r=-0.19$ ,  $p=0.17$ ,  $n=52$ ; Female *A. carolinensis*:  $r=-0.18$ ,  $p=0.17$ ,  $n=57$ ; Male *A. sagrei*:  $r=-0.13$ ,  $p=0.37$ ,  $n=46$ ; Female *A. sagrei*:  $r=-0.26$ ,  $p=0.06$ ,  $n=48$ ).

Male *A. carolinensis* ranged in mass from 1.6 to 7.2g (n=52). Female *A. carolinensis* ranged in mass from 1.3 to 3.8g (n=57) (Table 2). Male *A. sagrei* ranged in mass from 1.7 to 7.8g (n=46). Female *A. sagrei* ranged from 1.3 to 3.3g (n=48) (Table 2). There was a significant difference in mass between sexes for both species, males of *A. carolinensis* and *A. sagrei* are heavier than females (Student's T Test: *A. carolinensis*:  $t=7.5$ ,  $df=64.1$ ,  $p<0.0001$ ; *A. sagrei*:  $t=12.7$ ,  $df=51.2$ ,  $p<0.0001$ ). There was a significant difference in mass among populations of *A. carolinensis* (One way ANOVA, Males:  $F_{(3,48)}=20.3$ ,  $p<0.0001$ ; Females:  $F_{(3,53)}=26$ ,  $p<0.0001$ ). Individuals from Savannah, GA (females and males) were significantly heavier than those of other populations (Tukey's HSD,  $p<0.05$ ). There was also a significant difference in mass between populations of *A. sagrei* (One way ANOVA, Males:  $F_{(3,42)}=4.3$ ,  $p=0.009$ ; Females:  $F_{(3,44)}=16.9$ ,  $p<0.0001$ ) with males from the combined southernmost population significantly lighter than the males from Plant City and females from the southernmost population

significantly lighter than those from the other three populations (Tukey's HSD,  $p < 0.05$ ). Both male and female of *A. carolinensis* and *A. sagrei* exhibit a latitudinal gradient in mass as evidenced by the largest individuals from each of the four sites, ranging from 3.7g and 2.2g for males and females of *A. carolinensis*, respectively, from South Florida to 7.2g and 3.8g for males and females from Savannah, GA, respectively. Male and female *A. sagrei* ranged from 6.2g and 2.3g from South Florida, to 7.6g and 3.3g from Savannah, GA respectively. Summary statistics for all field variables can be found in Table 2.

Mean values of FCTMin for all four populations of *A. carolinensis* and *A. sagrei* can be found in Table 3. There was a significant correlation between FCTMin and mass for females of both species, with heavier individuals having lower FCTMin (*A. carolinensis*:  $r = -0.29$ ,  $p = 0.02$ ,  $n = 57$ ; *A. sagrei*:  $r = -0.26$ ,  $p = 0.04$ ,  $n = 48$ ), but not for males of either species (*A. carolinensis*:  $r = -0.21$ ,  $p = 0.12$ ,  $n = 52$ ; *A. sagrei*:  $r = -0.13$ ,  $p = 0.37$ ,  $n = 46$ ). There was a significant interaction between the effect of size and sex on weight of males and females of both species (Full Factorial ANOVA, sex\*svl: *A. carolinensis*  $F_{(1,105)} = 16.3$ ,  $p < 0.0001$ ; *A. sagrei*  $F_{(1,90)} = 23.3$ ,  $p < 0.0001$ ; Figure 2). The weight relative to the length of the individual (BCI), a measure of the energetic state of the individual (Schulte-Hostedde *et al.* 2005), was found to be lower in males of *A. carolinensis* than in females (ANCOVA, Sex:  $F_{(1,106)} = 21.7$ ,  $p < 0.0001$ ), but was not different between sexes in *A. sagrei* (ANCOVA, Sex:  $F_{(1,91)} = 3.04$ ,  $p < 0.08$ ). BCI was significantly related to FCTMin only in female *A. carolinensis* ( $r = -0.28$ ,  $p = 0.03$ ,  $n = 57$ ); individuals in better condition exhibited lower FCTMin. The other field variables were not significantly related to FCTMin. Because body mass affects cold tolerance, and because the two species vary in mass in these interspecific comparisons, analyses of covariance with body mass as the covariate were performed to look for differences between species, sex and population. A test of homogeneity of

regression could not detect interactions between the mass (the covariate) and species, sex or population (Species:  $F=0.05$ ,  $df=1$ ,  $p=0.81$ ; Sex:  $F=1.46$ ,  $df=1$ ,  $p=0.22$ ; Population:  $F=1.64$ ,  $df=3$ ,  $p=0.18$ ). *Anolis sagrei* had a significantly higher FCTMin than *A. carolinensis* (ANCOVA,  $F_{(1,200)} = 91.17$ ,  $p<0.0001$ ). Females of both species had significantly higher FCTMin than males (ANCOVA,  $F_{(1,200)} = 4.27$ ,  $p=0.04$ ). FCTMin differed among populations (ANCOVA,  $F_{(3,198)} = 16.05$ ,  $p<0.0001$ ). A Full Factorial ANOVA could not detect interactions between sex (sex\*species:  $F_{(1,187)}=2.6$ ,  $p=0.1$ ; sex\*population:  $F_{(3,187)}=3.39$ ,  $p=0.93$ ) in the FCTMin but detected an interaction between species and population, (Species\*Population:  $F_{(3,187)}=4.13$ ,  $p=0.007$ ). A follow up post hoc for the interaction effect revealed that for *A. carolinensis* the FCTMin of the southernmost population was significantly higher than that of the population from Savannah, GA, but doesn't differ from that of Plant City, FL; the FCTMin of individuals from Plant City, FL did not differ from that of St. Augustine, FL, but was significantly higher than that of Savannah, GA (Tukey HSD $<0.05$ ). For *A. sagrei* the combined southernmost populations had significantly higher FCTMin than the other populations; the FCTMin of the populations from Plant City, FL and St. Augustine, FL were not significantly different; and the population from Savannah had significantly lower FCTMin compared to the other populations (Tukey's HSD  $p<0.05$ ). Thus, the cold tolerances of both species differed in a fashion unrelated to their mass and a geographic cline in FCTMin is evident (Figure 3).

January was the coldest month of the year in all five localities sampled, followed by February and December, respectively (One way ANOVAS all  $p$ 's  $<0.0001$ , Tukey HSD  $<0.05$ ). As latitude increases, average minimum and maximum temperature decreases throughout the year (Table 4) and during the coldest months of the year (December-February), maximum number of consecutive days, maximum number of runs of 2 or more consecutive days and total

number of days with minimum daily temperature  $\leq 0^{\circ}\text{C}$  increases (Table 5). January Mean (2007-2011) Minimum Temperature (MMNT), Extreme Minimum Temperature (EMNT) and Number of days with minimum temperature equal or below  $0^{\circ}\text{C}$  (DT0 $^{\circ}$ ) were found to be significantly related to FCTMin in both sexes of both species. Samples from localities with lower MMNT and EMNT and larger DT0 $^{\circ}\text{C}$  exhibited significantly lower mean FCTMin. These climatic variables are also correlated with BCI for female and male *A. carolinensis* and female *A. sagrei*. Only EMNT was significantly related to BCI in male *A. sagrei*. Total Precipitation for January through July of 2011 (TPCP) was found to be significantly related to FCTMin only in male and female *A. carolinensis*; populations from localities exhibiting higher TPCP exhibited higher mean FCTMin. TPCP was found to be significantly related to BCI for male *A. sagrei* only. Results of the Pearson correlations are shown in Table 6 and descriptive statistics for Climatic variables in Table 7.

#### *Laboratory study-*

The LCTMin was found to be unrelated to mass or other field variables in the case of *A. carolinensis* (Pearson correlations:  $p > 0.67$ ,  $n = 30$ ). For *A. sagrei*, however, FCTMin was found to be related to LCTMin; populations that exhibited higher cold tolerance in the field also exhibited higher cold tolerance in the laboratory ( $r = 0.43$ ,  $p = 0.005$ ,  $n = 38$ ). A repeated measures ANOVA testing for the effect of acclimation on the cold tolerance with species, sex and populations as the effects and FCTMin and LCTMin as the dependent variables revealed that acclimation had a significant effect on cold tolerance: lower CTMin were noted in both species, sex and population after acclimation (acclimation:  $F_{(1,63)} = 138.2$ ,  $p < 0.0001$ ). The effect of acclimation did not depend upon species or sex (acclimation\*species:  $F_{(1,63)} = 1.75$ ,  $p = 0.19$ ; acclimation\*sex:  $F_{(1,63)} = 0.18$ ,  $p = 0.66$ ), but populations did differ in their responses to acclimation

(acclimation\*population:  $F_{(1, 63)}=1.75$ ,  $p=0.0018$ ). All three populations of both species tended to converge to a similar LCTMin of an average of 6.2°C after acclimation despite their different FCTMin and there was significantly less variation in mean LCTMin values than in mean FCTMin values (Brown-Forsythe:  $F_{(1,4)}=9.48$ ,  $p= 0.03$ ).

There was a significant difference between species in the LCTMin after acclimation; *A. carolinensis* exhibited lower LCTMin than *A. sagrei* (Factorial ANOVA:  $F_{(1, 56)}=56.6$ ,  $p<0.0001$ ) and the effect of sex on LCTMin did not depend upon population (Factorial ANOVA species\*population:  $F_{(2,56)}=4.17$ ,  $p =0.4$ ). Neither species differed in LCTMin with respect to sex (Factorial ANOVA  $F_{(1,56)}=2.29$ ,  $p=0.31$ ) or population (Factorial ANOVA  $F_{(2,56)}=127.9$ ,  $p=0.76$ ), (Table 3, Figure 4).

## Discussion

Michaud & Echternacht (1995) noted a south-to-north increase in size of female *A. carolinensis* sampled at localities from East Tennessee to South Florida, and Goodman (2010) confirmed that finding among four populations along a similar transect. The current study revealed a latitudinal trend in SVL for male *A. carolinensis* and in mass for males and females of both species. There was, however, no latitudinal pattern for female *A. carolinensis* as reported by Michaud & Echternacht (1995). Goodman *et al.* (in review) subsequently sampled *A. carolinensis* across the entire range of the species and found a south-to-north size increase only among the easternmost populations sampled, those which approximated the transects of Michaud and Echternacht (1995) and Goodman (2010). In addition, Goodman *et al.* (in review) found the largest population means occurring in the southwestern part of the range, in Louisiana and coastal Texas. This pattern disappeared, however, if populations from peninsular Florida were removed from the analysis. In fact, Goodman (2009) and Goodman *et al.* (in review), found an east-to-west longitudinal increase in mean size. It may be that at least a part of the disparity between the results of this study and those of Michaud & Echternacht (1995), Goodman (2009, 2010) and Goodman *et al.* (in review) are due to the extreme drought that the southeastern United States was experiencing in 2010 and 2011 as well as other factors discussed by Goodman *et al.* (in review). In the southeast, Florida and Georgia have been especially hard hit by the drought and the reduction in the prey base exploited by the lizards may have been reduced, affecting the size of *A. carolinensis* in the region. In addition, at the time that the earlier studies were conducted, *A. sagrei* had only recently arrived in northern Florida and Georgia and had yet to have a significant competitive affect on *A. carolinensis*. It is clear that determinates of body size in *A.*

*carolinensis* deserves considerably more study. It is unfortunate that equivalent size information on populations of *A. sagrei* in northern Florida and Georgia are not available.

The lack of a relationship between SVL and FCTMin, is consistent with the findings of other investigators (McConnachie *et al.* 2007, Wilson & Echternacht, 1987, Yang *et al.* 2008, but see Labra *et al.* 2009), but the finding that mass affected FCTMin, though only in female *A. carolinensis* and *A. sagrei*, has not been previously reported (e.g., Spellerberg & Spellerberg 1972, Wilson & Echternacht 1987), although in these studies a small range of adult body sizes were used and in the latter case only male lizards were studied. McConnachie *et al.* (2007) reported a lack of relationship between mass and CTMin after acclimation of male and female *Pseudocordylus melanotus* (Sauria:Cordylidae), but the frequent failure to investigate this relationship or even to report the sex of the study organisms makes difficult to see how widespread this relationship is. Furthermore, when interspecific or interpopulation comparisons of thermal tolerance are made, it is very important to account for the potential influences of mass and body size on thermal tolerances.

FCTMin varied among species, sex and location for the two species of anoles in this study, and exhibited a latitudinal cline (Figure 3). This variation appears to be strongly related to the environmental variables to which the lizards have been exposed during their lives (thermal acclimation), such as the extreme minimum temperature and the average minimum temperature of the coldest month of the year that they have experienced. *Anolis carolinensis* and *A. sagrei* produce a new cohort a year, during the breeding season (March-August; Lee *et al.* 1989, Gordon 1956) of the year following that in which they themselves were produced and, since the estimated longevity of these lizards in the wild is approximately a year (Oliver 1955, Gordon 1956, Cox & Calsbeek 2009) we can assume that most of the individuals in our study were first

year adults that experienced their lowest environmental temperatures during the winter of 2010. The difference in cold tolerance is greater between individuals from Savannah, GA and the southern population (Naples and east of Punta Gorda, FL) which is consistent with a greater difference between those sites in the minimum temperatures experienced during the winter. In addition, the least difference in cold tolerance found between individuals from Plant City and St. Augustine, FL, reflects the lack of significant difference in the environmental temperatures between those sites during the winter (Figure 5, Table 7).

The difference in FCTMin between females and males of both species could be attributed to different thermal environments experienced by the individuals; both species studied have a marked sexual dimorphism (Preest 1994, Fitch 1976, Lee 1987) that has been associated with differential use of microhabitats by males and females (Butler *et al.* 2000). *Anolis sagrei* males have been found to use significantly higher perches than females (Shoener 1968) and although no significant differences in habitat use for female and male *A. carolinensis* have been detected (Jenssen & Nunez 1998), the structural niche profile could vary greatly depending on the variety of habitats in which the species is found. These different microhabitats could expose the lizards to different minimum temperatures during the winter that could affect their response to exceptionally cold temperatures.

The BCI can be used to estimate of the energetic state of an individual. Thus, it has been suggested that an animal in good condition (e.g., higher BCI) might have more energy reserves than an animal in poor condition (Schulte-Hostedde *et al.* 2005). In fish (walleye pollock, *Theragra chalcogramma*), an increase in cold tolerance and in survival has been noted in individuals with a higher BCI (Sogard & Olla 2000). In the present study, the mean BCI of female *A. sagrei* did not significantly differ from that of males whereas in *A. carolinensis*, the

female were in significantly better condition than males. Prior to the present study, the field study area in Florida had experienced an extended period of drought and two unusually cold winters, which may have resulted in a reduced prey resource base. If so, the results of this study may suggest that, while the impact of a reduction in prey may have been to reduce BCI of both *A. sagrei* and *A. carolinensis*, its greatest impact has been on female *A. carolinensis*. The lower cold tolerance (higher CTMin) observed in female *A. carolinensis* as compared to males might be attributed to an elevated cost of reproduction by females resulting from reduced energy acquisition and allocation for maintenance (Cox & Calsbeek 2009). Currently, no long term data have been published on population trends for either *A. sagrei* or *A. carolinensis*, nor their prey base, which would allow an assessment of the impact of climate change on BCI and possible secondary effects on population density.

Acclimation has been found to significantly affect thermal tolerances of lizards (Lowe & Vance 1955, Kour & Hutchison 1970), and the greatest degree of acclimation has been found to be achieved at a temperature of 1°C below the mean FCTmin (Spellerberg 1972). Since acclimation affects cold tolerance and it can be attained very quickly, within 48 hours for most species (Spellerberg & Spellerberg 1972), acclimation to a common temperature is needed to be able to detect differences in thermal tolerances not caused by the thermal histories that the individuals have experienced in the field. After accounting for the thermal histories of the individuals, we found no differences in the critical thermal minimum between populations of either species which suggests that for both species critical thermal minimum shows thermal acclimation. This response has also been documented for two subspecies of the lizard *Mabouya striata* (Scincidae), *Stellio stellio* (Agamidae), ten species of the genus *Liolaemus* (Iguanidae) that live at different altitudes (Patterson 1991, Hertz & Nevo 1981, Carothers *et al.* 1997) and a

number of species of Australian reptiles (Spellerberg 1972). *Anolis carolinensis* had a mean CTMin of  $4.8^{\circ}\text{C} \pm 0.2$ , a value considerably lower than what was previously reported for this species at the northern limit of the distribution ( $7.41^{\circ}\text{C}$  from individuals from Tallassee, TN; Wilson & Echternacht 1987) and *A. sagrei* had a mean CTMin of  $7.6^{\circ}\text{C} \pm 0.2$ , a value higher than what was previously found for *A. sagrei* in northern Florida (Lake City; approximately  $5.2^{\circ}\text{C}$  in individuals acclimated at  $15^{\circ}\text{C}$ ; Denesha 2006). However, caution must be exercised in comparing our measures to those collected in other studies since experimental protocols differ slightly. We used the temperature at which the lizards stopped walking as the endpoint of CTMin rather than the loss of righting response (LRR) endpoint, which may reflect more directly the limits of locomotor performance due to difficulties with *A. sagrei* explained in the methods, but our measures of the cold tolerance should be considered as conservative. We also acclimated our animals following the methods of Spellerberg (1973) which requires the acclimation of the individuals at  $1^{\circ}\text{C}$  below the field CTMin and provides the greatest degree of acclimatability and repeatability for the CTMin (Spellerberg & Spellerberg 1972). Many studies of cold tolerance in reptiles conducted since Spellerberg's 1972 publication fail to measure the FCTMin of individuals and acclimate the lizards to a temperature chosen arbitrarily. Since acclimation temperature is known to affect CTMin values obtained following acclimation (Patterson 1991, Tsuji 1988, Yang *et al.* 2008) and since values measured in lizards acclimated to similar temperatures will be necessary for accurate comparisons, the basis for the choice of an acclimation temperature must be justifiable and reported.

Wilson and Echternacht (1987) reported significant differences in LCTMin in *A. carolinensis* following acclimation between populations from Florida and Tennessee and between populations from south Georgia and Tennessee, but they failed to find significant

differences between populations from Florida and south Georgia. We also didn't see differences between our populations, and our latitudinal gradient only included individuals from Florida and Georgia, this lack of differentiation between populations could be due to lack of genetic isolation between the populations or that the extreme minimum temperatures experienced during the winter are not comparable to the ones experienced in Tallassee, TN. This locality is considered to be in a different climate zone than south Georgia and Florida based on the average annual extreme minimum temperature over a 30-year period, the average for Tallassee is 12.5°C lower than the average of the values from Florida and south Georgia (USDA 2012). However, the persistence of an interspecific difference in all the populations in cold tolerance after the long period of laboratory acclimation suggests the presence of a genetic component in this characteristic.

Lower critical thermal minimum has been suggested to influence distribution and range size in ectothermic organisms (Prieto & Whitford 1971, Spellerberg 1972, 1973, Spellerberg & Spellerberg 1972, Greer 1980) since lizards can behaviorally avoid dangerously high temperatures but not as readily avoid those that are dangerously low (Heatwole *et al.* 1969, Spellerberg & Spellerberg 1972, Carothers *et al.* 1997). In some cases, however, it has been suggested that it is the ability to make rapid adjustments to the local environments which can set the distributional limits of a species (Brattstrom 1965). This proposed relationship between thermal acclimatization ability and geographic range size in ectotherms is termed the Brattstrom hypothesis (Gaston *et al.* 2009). *Anolis carolinensis* has a greater cold tolerance than *A. sagrei*. Considering that both species have a Caribbean origin (Williams 1969), this difference in cold tolerance could be due to the greater time that *A. carolinensis* has had to adapt to the lower temperatures experienced during the winter months at higher latitudes.

Is apparent that both *A. sagrei* and *A. carolinensis* exhibit thermal acclimatization to a wide range of thermal conditions which has the potential of enhancing their ability to colonize the temperate zone. However, adaptive shifts in the thermal limits occur over longer time scales (Anguilleta 2002) and this could explain why *A. carolinensis* exhibits a greater cold tolerance after acclimation than *A. sagrei* in all of the populations studied. *Anolis carolinensis* has been reported in numerous cave deposits dating from the late Pleistocene (Holman 1995), whereas *A. sagrei* was first reported on the mainland of peninsular Florida about 70 years ago (Lee 1985). The tendency for species from cooler environments to have lower CT<sub>Min</sub> has already been described for several species of lizards (Bennet & John-Alder 1986; Brown 1996; Spellerberg, 1972, 1973, 1975; Spellerberg & Spellerberg 1972; Prieto & Whitford 1971; Tsuji 1988), reflecting the latitude or altitude and thus the climates in which the species naturally occur. Several species in the genus *Anolis* also show climate-related among-population differentiation of critical thermal limits (Denesha 2006, Hertz 1981, Hertz & Huey 1981, Hertz *et al.* 1979, Wilson & Echternacht 1987).

Whether the differentiation in critical thermal limits between populations has a genetic component or is the result of thermal acclimatization (phenotypic plasticity), as it seems the case in the among population differentiation for *A. carolinensis* and *A. sagrei* reported here, any change in the critical thermal limit will directly affect the range of body temperatures under which locomotion remains possible, and thus may lead to a reduction in the frequency with which the animal is left immobile (depending on the direction of the change and the range of body temperatures experienced). This could have considerable implications for survival and could increase the ability of an invasive species to spread from a subtropical zone towards a temperate zone. *Anolis sagrei* is reported to have spread to South Carolina and North Carolina by

2006 and 2008, respectively, but in June of 2011 and May and June of 2012, in the course of this study, they were found to be absent at sites in South Carolina where they had been previously reported. These populations may have been extirpated during the unusually cold winter of 2010 (Turnbough 2012) when mean minimum temperatures of  $-2.2^{\circ}\text{C}$  and  $-1.7^{\circ}\text{C}$  were recorded in Orangeburg and Colleton counties, respectively. This could mean that although *A. sagrei* has a remarkable capacity to acclimate to local conditions, it is close to its northern distributional limit. There may, however, be some behavioral traits that allow these lizards to escape lethally cold temperatures in places that reach freezing temperatures during the winter. Most of the *A. sagrei* found in northeast Georgia were found in close association with human constructions and were observed using the heating/AC systems as refuges during the summer. If this behavior is present during the winter months is likely that they are not experiencing extreme cold temperatures, reducing the need for an adjustment in CTMin. This might allow the establishment and survival of populations in suitable artificial habitat to the north.

To further explore how the ability to adjust lower critical thermal limits influences geographic distribution in *Anolis sagrei*, we need to determine if this ability is heritable, if it increases survivorship under natural conditions, and if phenotypic plasticity in tolerance to low temperatures increases fitness in nature. These studies may be particularly valuable in understanding the role of environmental variation in the evolution of plasticity and its influence in distribution of ectotherms.

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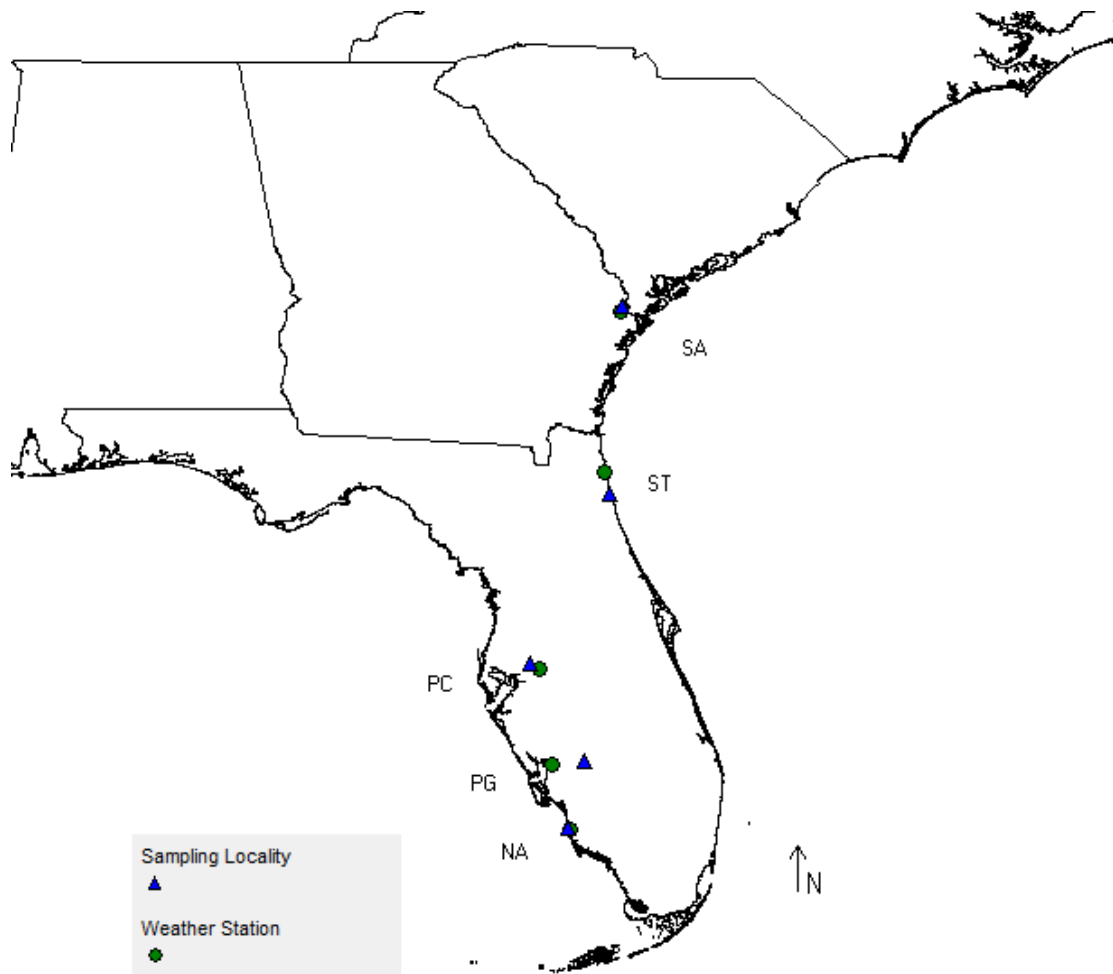
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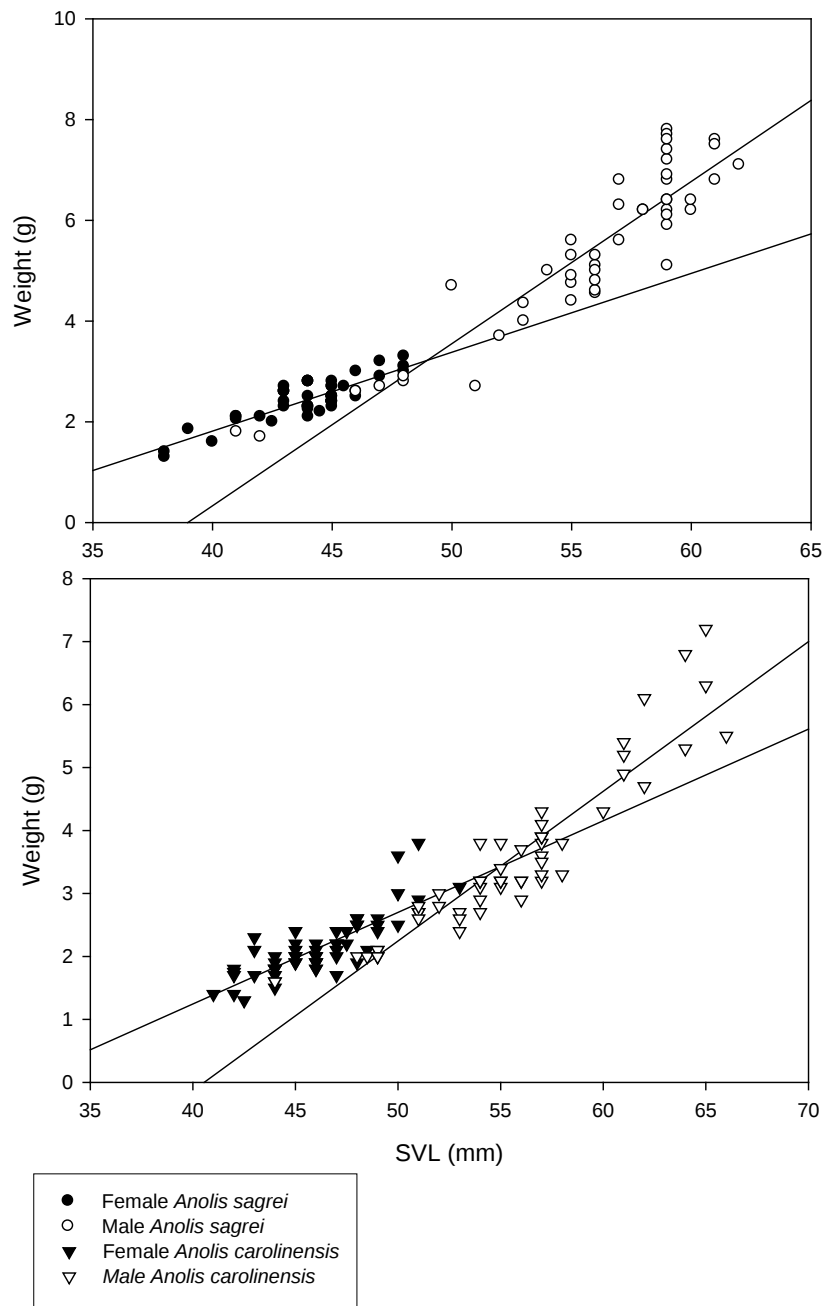
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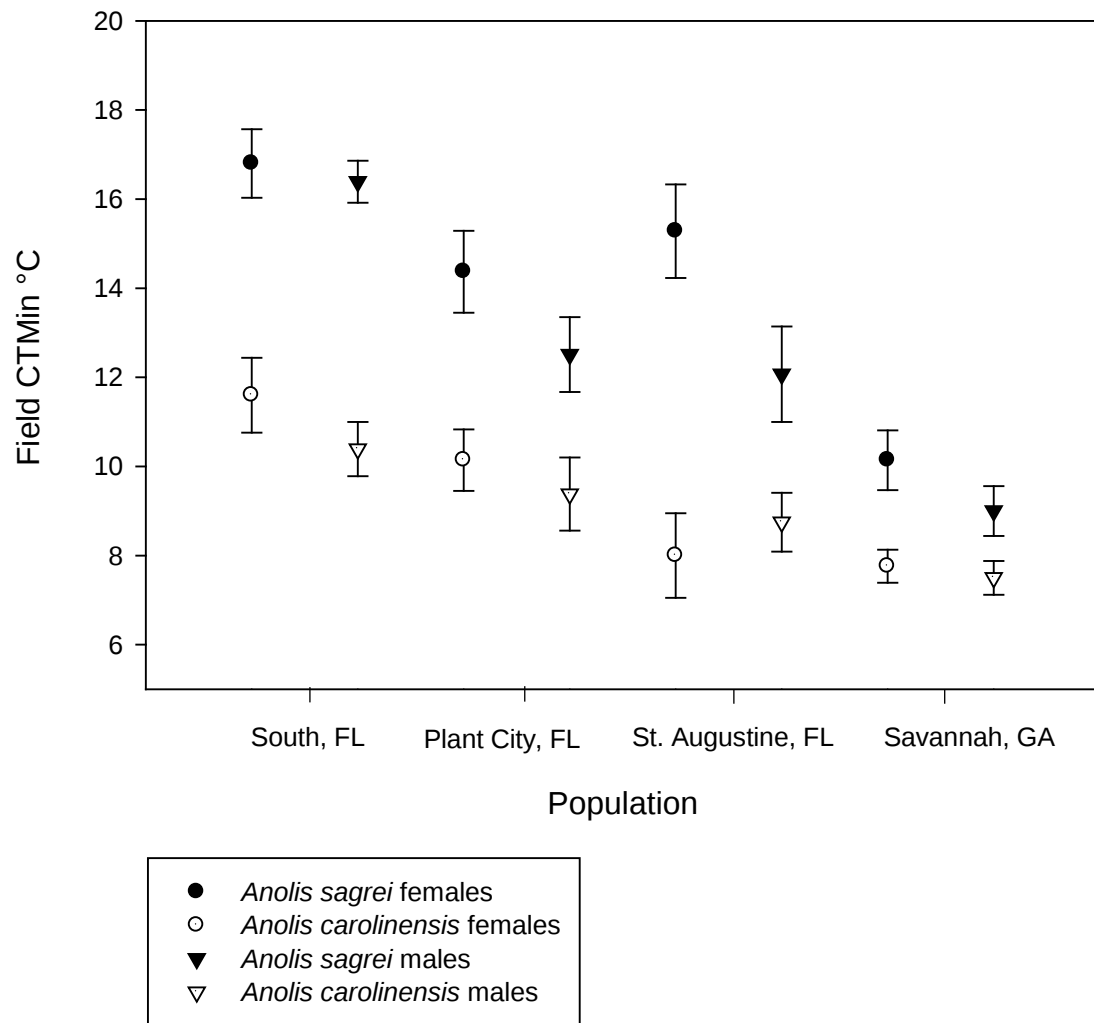
## **Appendix**



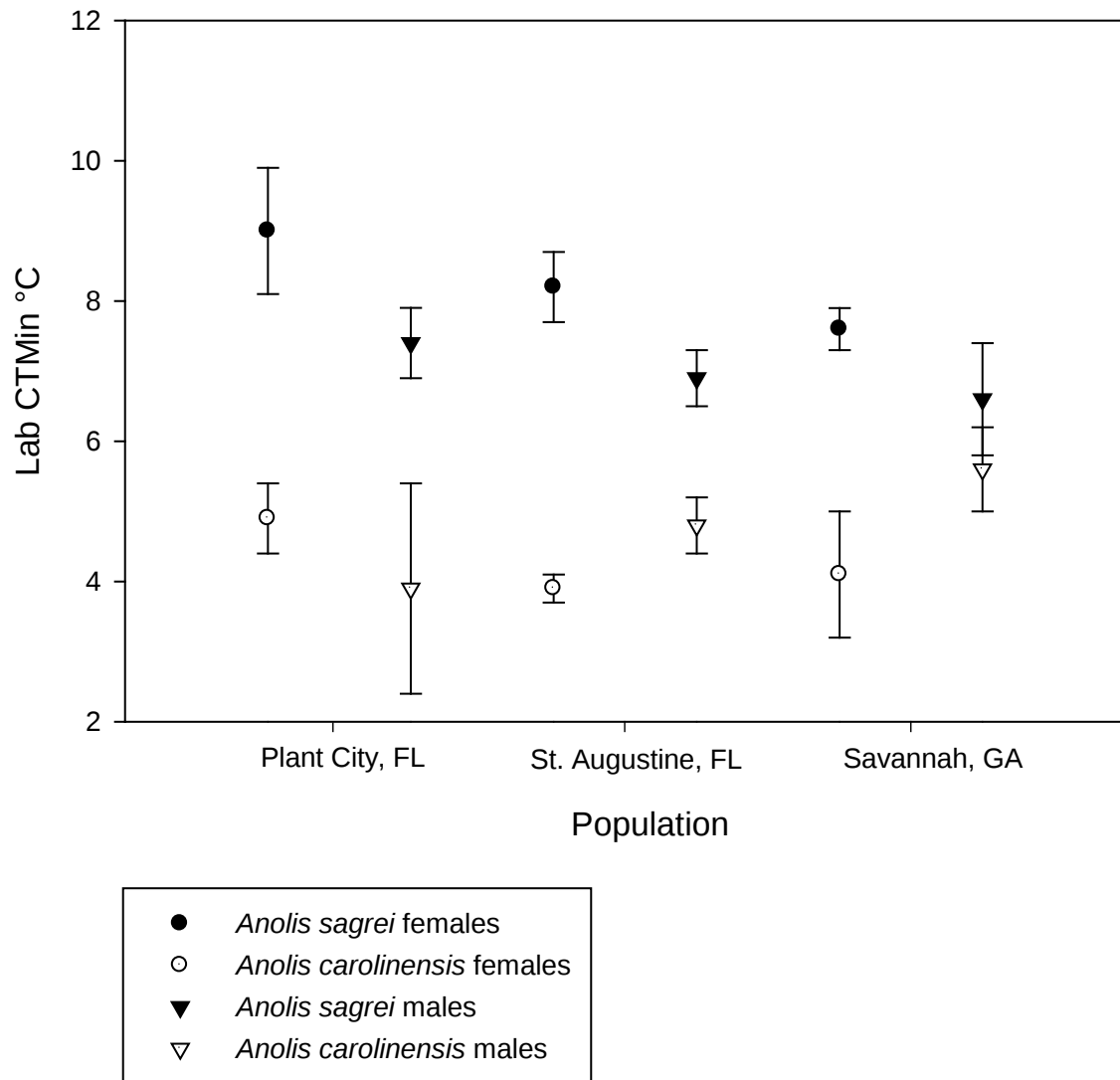
**Figure 1.** Sampling localities, Naples, FL (NA), Punta Gorda, FL (PG), Plant City, FL (PC), St. Augustine, FL (ST) and Savannah, GA (SA), for *Anolis sagrei* and *Anolis carolinensis* and location of the nearest NOAA weather station from which five year temperature and precipitation data were obtained. See Table 1 for further details.



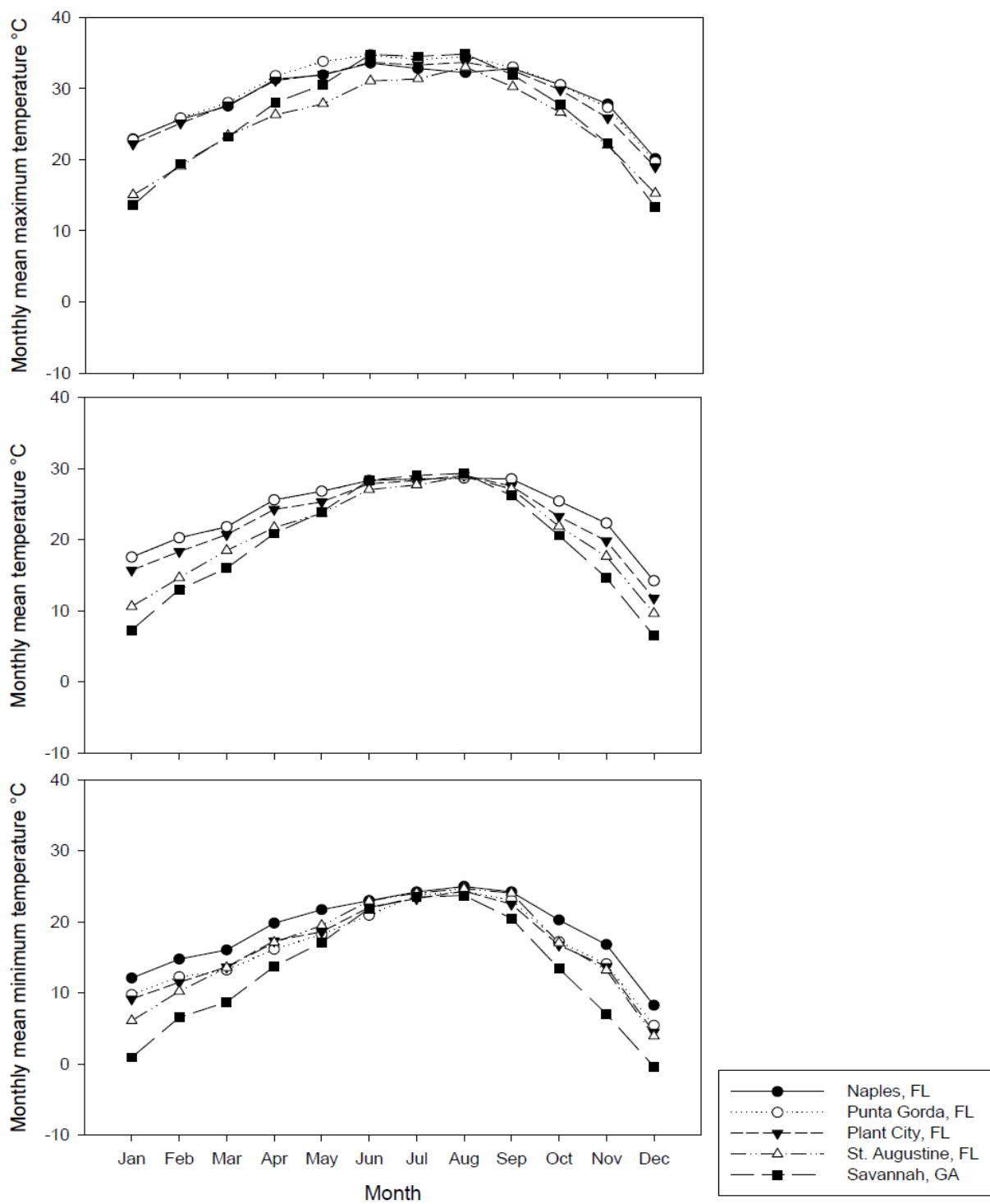
**Figure 2.** Interaction plot for the effect of SVL on mass between females and male *Anolis sagrei* and *Anolis carolinensis*.



**Figure 3.** Field CTMin (°C) for females and males of *A. sagrei* and *A. carolinensis*. The FCTMin decreases as the latitude of the population increases.



**Figure 4.** Laboratory Critical Thermal Minima (LCTMin) for females and males of *Anolis sagrei* and *Anolis carolinensis*. There is not a significant difference in LCTMin between populations of both species, but LCTMin is significantly lower in *A. carolinensis* in all three populations studied.



**Figure 5.** Five year average monthly minimum, mean and maximum temperature for the five localities sampled.

**Table 1.** Sampling localities for *Anolis sagrei* (AS) and *Anolis carolinensis* (AC), which species were sampled at each, and location of the nearest NOAA weather station from which five year temperature and precipitation data were obtained. See Figure 1 for a visual representation.

| Sampling Locality                  | Latitude     | Longitude       | Elevation (m) | Species Sampled |    | Nearest NOAA Weather Station       | Latitude | Longitude | Elevation (m) |
|------------------------------------|--------------|-----------------|---------------|-----------------|----|------------------------------------|----------|-----------|---------------|
|                                    |              |                 |               | AS              | AC |                                    |          |           |               |
| Georgia: Vicinity of Savannah      | 32°00'19'' N | 81°16'49.3'' W  | 2             | x               | x  | Savannah INTL AP 097847/03822      | 32°08' N | 81°13' W  | 14            |
| Florida: Vicinity of St. Augustine | 30°01'20'' N | 81°19' 59.2'' W | 17            | x               | x  | Jacksonville Beach 084366/99999    | 30°17' N | 81°24' W  | 3             |
| Florida: Plant City                | 28°01'29''N  | 82°09'02'' W    | 14            | x               | x  | Plant City 087205/99999            | 28°01' N | 82°09' W  | 36.5          |
| Florida:48 km E of Punta Gorda     | 26°56'43''N  | 81°36'50'' W    | 22            | o               | x  | Punta Gorda 4(mi) ESE 087397/99999 | 26°55' N | 82°00' W  | 6             |
| Florida: Vicinity of Naples        | 26°10'30''N  | 81°48'09'' W    | 7             | x               | o  | Naples MUNI AP 086076/12897        | 26°09' N | 81°47' W  | 2.7           |

**Table 2.** Descriptive statistics for field habitat variables of *Anolis sagrei* and *Anolis carolinensis* collected from their southernmost localities and Plant city, FL.

| Species              | South, FL                |                          |                          |                          | Plant City, FL            |                           |                          |                          |
|----------------------|--------------------------|--------------------------|--------------------------|--------------------------|---------------------------|---------------------------|--------------------------|--------------------------|
|                      | <i>A. sagrei</i>         |                          | <i>A. carolinensis</i>   |                          | <i>A. sagrei</i>          |                           | <i>A. carolinensis</i>   |                          |
| Sex                  | F<br>(11)                | M<br>(13)                | F<br>(16)                | M<br>(16)                | F<br>(16)                 | M<br>(14)                 | F<br>(20)                | M<br>(13)                |
| <b>SVL (mm)</b>      | 42.7+2.28<br>(38-65)     | 53.9+1.07<br>(46-59)     | 44.2+0.46<br>(41-47)     | 51.9+0.88<br>(44-57)     | 44.7+0.41<br>(42-48)      | 57.1+1.33<br>(47-62)      | 45.8+0.46<br>(42-49)     | 54.3+1.07<br>(44-58)     |
| <b>Weight (g)</b>    | 1.8+0.1<br>(1.3-2.3)     | 4.3+0.3<br>(2.6-6.2)     | 1.8+0.06<br>(1.3-2.2)    | 2.6+0.14<br>(1.6-3.7)    | 2.5+0.06<br>(2.1-3.1)     | 6.2+0.45<br>(2.7-7.8)     | 2.0+0.05<br>(1.4-2.5)    | 3.2+0.2<br>(1.6-4.1)     |
| <b>BT (°C)</b>       | 32.1+0.95<br>(24.8-35.4) | 33.1+0.5<br>(29.5-36.2)  | 34+0.48<br>(29-36.4)     | 34.1+0.57<br>(30.2-38)   | 29.9+0.65<br>(26-34.8)    | 28.9+0.67<br>(25.4-35)    | 30.6+0.44<br>(25.8-34.6) | 30.8+0.68<br>(27.6-35.6) |
| <b>AT1cm (°C)</b>    | 30.9+0.73<br>(26-34)     | 31.5+0.65<br>(27.1-35)   | 32.6+0.53<br>(28.6-35.8) | 32.6+0.71<br>(27.2-37.4) | 28.5+0.59<br>(24.6-32.8)  | 27.8+0.76<br>(24.6-34)    | 29.4+0.46<br>(25.4-34.2) | 29.2+0.72<br>(26.4-34.6) |
| <b>AT1m (°C)</b>     | 30.4+0.88<br>(25.2-33.4) | 31.3+0.67<br>(27-34.2)   | 31.9+0.46<br>(28-34.2)   | 32.2+0.58<br>(27.6-35)   | 28.7+0.59<br>(24.8-33)    | 27.2+0.7<br>(24.2-32.4)   | 29.3+0.42<br>(25.4-33)   | 28.9+0.7<br>(26.3-34)    |
| <b>RH%</b>           | 61.4+0.48<br>(60.2-65.2) | 63.6+2.05<br>(60.2-88.1) | 68.7+2.92<br>(56.8-92)   | 69+2.88<br>(52-80)       | 83.3+2.27<br>(64.2-92)    | 85.5+1.73<br>(67.7-92)    | 81.3+2.66<br>(62.3-92)   | 74.3+3.51<br>(46.3-92)   |
| <b>PH (mm)</b>       | 203+103.7<br>(0-1179)    | 261.1+80<br>(0-1140)     | 760.3+125.5<br>(0-2290)  | 1078+104.7<br>(390-1910) | 870.3+141.7<br>(260-2280) | 902.2+153.1<br>(320-2350) | 811+163.5<br>(0-2470)    | 1322+251.9<br>(0-3300)   |
| <b>PD (mm)</b>       | 44+33.9<br>(0-375.2)     | 15.5+10.6<br>(0-135.2)   | 29+14.9<br>(0-247)       | 16.3+7.2<br>(0-120.6)    | 157.1+51.2<br>(0-611.4)   | 146+53.2<br>(0-676.7)     | 42.5+20.6<br>(0-342.8)   | 51.3+30.6<br>(0-390)     |
| <b>Veg Cover (%)</b> | 89.7+5.1<br>(51-100)     | 80.8+8.1<br>(0-100)      | 71.8+8<br>(6.2-98.9)     | 55.7+11.3<br>(0-100)     | 94.5+3.4<br>(51-100)      | 97.9+1.2<br>(82.2-100)    | 89+4.7<br>(14.5-100)     | 91+5.6<br>(25-100)       |

Data are expressed as mean  $\pm$  SE and range. Numbers in parenthesis are sample sizes.

Table 2 continued.

| Species              | St. Augustine, FL        |                          |                          |                          | Savannah, GA             |                          |                           |                            |
|----------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|---------------------------|----------------------------|
|                      | <i>A. sagrei</i>         |                          | <i>A. carolinensis</i>   |                          | <i>A. sagrei</i>         |                          | <i>A. carolinensis</i>    |                            |
| Sex                  | F<br>(11)                | M<br>(10)                | F<br>(10)                | M<br>(12)                | F<br>(10)                | M<br>(9)                 | F<br>(11)                 | M<br>(11)                  |
| <b>SVL (mm)</b>      | 44.8+0.29<br>(44-47)     | 56+1.71<br>(42-60)       | 46.7+0.78<br>(44-51)     | 56.3+1.04<br>(51-62)     | 44.9+0.54<br>(43-48)     | 57+0.62<br>(55-60)       | 49.4+0.52<br>(47-53)      | 61.5+1.13<br>(55-66)       |
| <b>Weight (g)</b>    | 2.4+0.08<br>(2.1-3)      | 5.6+0.53<br>(1.7-7.4)    | 2.1+0.09<br>(1.8-2.7)    | 3.5+0.25<br>(2.4-5.2)    | 2.7+0.1<br>(2.3-3.3)     | 5.6+0.3<br>(4.8-7.6)     | 2.8+0.14<br>(2.4-3.8)     | 5.3+0.39<br>(3.2-7.2)      |
| <b>BT (°C)</b>       | 32.7+0.42<br>(29.6-34.4) | 32.8+0.34<br>(31-34.4)   | 33.2+0.46<br>(30.4-35.2) | 32.4+0.47<br>(30.2-35.8) | 32.3+1.31<br>(25.6-38.2) | 31+1.29<br>(25.6-36.6)   | 30.9+1.05<br>(26.6-37.2)  | 33.6+0.62<br>(28.6-36)     |
| <b>AT1cm (°C)</b>    | 32+0.49<br>(28.8-34)     | 32.1+0.67<br>(29.4-36)   | 32.7+0.81<br>(29.8-38.4) | 30.8+0.49<br>(28.4-33.6) | 31.3+1.51<br>(24.2-36.4) | 30.8+1.51<br>(25.4-38.4) | 29.8+1.15<br>(25.4-37)    | 32.9+0.8<br>(27.6-36)      |
| <b>AT1m (°C)</b>     | 31.2+0.5<br>(28-33.2)    | 31.5+0.73<br>(29.6-37)   | 31.4+0.42<br>(29.6-34.2) | 30.2+0.49<br>(27-32.8)   | 31.5+1.35<br>(25.6-36.6) | 30.0+1.36<br>(24.8-36)   | 29.3+1.0<br>(25.4-36)     | 33.2+0.81<br>(27.4-37.2)   |
| <b>RH%</b>           | 61.3+1.39<br>(52.8-72.4) | 59.8+0<br>(59.8-59.8)    | 69+2.65<br>(59.8-79.1)   | 66.2+3.56<br>(52.8-100)  | 75.2+3.7<br>(47.7-88.6)  | 80.1+2.44<br>(72.2-88.6) | 83.2+2.25<br>(64.8-92)    | 65.8+5.52<br>(39.6-85)     |
| <b>PH (mm)</b>       | 804.8+235.6<br>(0-2020)  | 1247+154.4<br>(260-2160) | 605.5+167<br>(0-1500)    | 868.5+123.1<br>(0-1330)  | 444.6+123<br>(140-1286)  | 778.8+237.1<br>(0-1810)  | 686.3+113.1<br>(220-1360) | 1291.8+208.3<br>(550-2600) |
| <b>PD (mm)</b>       | 72.1+23<br>(0-207)       | 93+31.6<br>(0-340)       | 91.2+41.2<br>(0-330)     | 24.4+10.3<br>(0-110)     | 63.6+34.3<br>(0-356)     | 108.3+54.5<br>(0-365)    | 4+2.6<br>(0-24)           | 37.8+7.9<br>(0-80)         |
| <b>Veg Cover (%)</b> | 85.8+7.5<br>(20.8-100)   | 93.3+2.2<br>(81.2-100)   | 82.1+10<br>(0-100)       | 73.2+11.3<br>(0-100)     | 93.4+2.7<br>(75-100)     | 80.9+9<br>(15.6-100)     | 66.6+11.2<br>(0-98.9)     | 93+3.5<br>(60.4-100)       |

Data are expressed as mean  $\pm$  SE and range. Numbers in parenthesis are sample sizes.

**Table 3.** Descriptive statistics for Field and Laboratory Critical Thermal Minima (CTMin) of *Anolis sagrei* and *Anolis carolinensis*.

| Population        | Field CTMin                       |                                  |                                  |                                 | Laboratory CTMin              |                              |
|-------------------|-----------------------------------|----------------------------------|----------------------------------|---------------------------------|-------------------------------|------------------------------|
|                   | <i>A. sagrei</i>                  |                                  | <i>A. carolinensis</i>           |                                 | <i>A. sagrei</i>              | <i>A. carolinensis</i>       |
|                   | Female                            | Male                             | Female                           | Male                            |                               |                              |
| Southern, FL      | 16.8 $\pm$ 0.77 (11)<br>13.8-22.3 | 16.3 $\pm$ 0.47 (13)<br>13-18.7  | 11.6 $\pm$ 0.84 (16)<br>7.3-19.9 | 10.3 $\pm$ 0.61 (16)<br>7-16.3  | -                             | -                            |
| Plant City, FL    | 14.3 $\pm$ 0.92 (16)<br>8.3-20.9  | 12.5 $\pm$ 0.84 (14)<br>8.2-18.4 | 10.1 $\pm$ 0.69 (20)<br>5.5-17.3 | 9.3 $\pm$ 0.82 (13)<br>5.8-15.1 | 8.04 $\pm$ 0.5 (15)<br>4.2-13 | 4.6 $\pm$ 0.5 (6)<br>2.4-6.5 |
| St. Augustine, FL | 15.2 $\pm$ 1 (11)<br>10.3-20.2    | 12 $\pm$ 1 (10)<br>7.8-18        | 8 $\pm$ 0.95 (10)<br>5.9-16      | 8.7 $\pm$ 0.66 (12)<br>6.4-14   | 7.5 $\pm$ 0.4 (11)<br>5.6-9.8 | 4.4 $\pm$ 0.3 (9)<br>3-7.2   |
| Savannah, GA      | 10.1 $\pm$ 0.67 (10)<br>8-15.2    | 9 $\pm$ 0.56 (9)<br>7.2-11.7     | 7.7 $\pm$ 0.37 (11)<br>5.8-9.4   | 7.5 $\pm$ 0.38 (11)<br>6.2-10.2 | 7.25 $\pm$ 0.4 (12)<br>3.5-9  | 5.3 $\pm$ 0.5 (11)<br>3-8.3  |

Data are expressed as mean  $\pm$  SE and range. Numbers in parenthesis are sample sizes.

**Table 4.** Mean Daily Minimum and Maximum temperature for each month for five years (2007-2011) for each of the field sites. Data represent means (SE). Different superscripts within each month indicate a significant difference ( $P<0.05$ ) between localities (ANOVA, Tukey's HSD)

|                            | Naples, FL               | Punta Gorda, FL           | Plant City, FL           | St. Augustine, FL        | Savannah, GA             | Effect of Locality <sup>a</sup> |
|----------------------------|--------------------------|---------------------------|--------------------------|--------------------------|--------------------------|---------------------------------|
| <b>Minimum Temperature</b> |                          |                           |                          |                          |                          |                                 |
| January                    | 12.6 (0.4) <sup>A</sup>  | 10.2 (0.4) <sup>B</sup>   | 9.3 (0.4) <sup>BC</sup>  | 7.7 (0.4) <sup>C</sup>   | 3.3 (0.4) <sup>D</sup>   | ***                             |
| February                   | 13.4 (0.3) <sup>A</sup>  | 11.2 (0.4) <sup>B</sup>   | 9.8 (0.4) <sup>BC</sup>  | 8.5 (0.4) <sup>C</sup>   | 4.2 (0.4) <sup>D</sup>   | ***                             |
| March                      | 15.3 (0.2) <sup>A</sup>  | 12.9 (0.3) <sup>B</sup>   | 12.4 (0.3) <sup>B</sup>  | 12.5 (0.3) <sup>B</sup>  | 8.2 (0.3) <sup>C</sup>   | ***                             |
| April                      | 18.2 (0.2) <sup>A</sup>  | 15.9 (0.2) <sup>B</sup>   | 15.6 (0.2) <sup>B</sup>  | 15.9 (0.2) <sup>B</sup>  | 12.2 (0.3) <sup>C</sup>  | ***                             |
| May                        | 21.3 (0.1) <sup>A</sup>  | 19 (0.2) <sup>C</sup>     | 19.2 (0.2) <sup>C</sup>  | 20.4 (0.1) <sup>B</sup>  | 17.5 (0.2) <sup>D</sup>  | ***                             |
| June                       | 23.6 (0.1) <sup>A</sup>  | 22.1 (0.1) <sup>C</sup>   | 22.1 (0.1) <sup>B</sup>  | 23 (0.1) <sup>C</sup>    | 21.9 (0.1) <sup>C</sup>  | ***                             |
| July                       | 24.3 (0.1) <sup>A</sup>  | 23.5 (0.09) <sup>BC</sup> | 23.2 (0.09) <sup>C</sup> | 23.7 (0.09) <sup>B</sup> | 22.6 (0.1) <sup>D</sup>  | ***                             |
| August                     | 24.9 (0.08) <sup>A</sup> | 24.2 (0.09) <sup>B</sup>  | 23.6 (0.08) <sup>C</sup> | 24.4 (0.1) <sup>B</sup>  | 23.4 (0.1) <sup>C</sup>  | ***                             |
| September                  | 24.1 (0.07) <sup>A</sup> | 22.9 (0.1) <sup>B</sup>   | 22.4 (0.1) <sup>B</sup>  | 23.7 (0.1) <sup>A</sup>  | 20.2 (0.2) <sup>C</sup>  | ***                             |
| October                    | 21.3 (0.2) <sup>A</sup>  | 19.4 (0.3) <sup>B</sup>   | 18.3 (0.3) <sup>B</sup>  | 19 (0.3) <sup>B</sup>    | 14 (0.4) <sup>C</sup>    | ***                             |
| November                   | 16.5 (0.3) <sup>A</sup>  | 14 (0.3) <sup>B</sup>     | 13.4 (0.3) <sup>B</sup>  | 13.4 (0.3) <sup>B</sup>  | 7.8 (0.3) <sup>C</sup>   | ***                             |
| December                   | 14.2 (0.3) <sup>A</sup>  | 11.8 (0.4) <sup>B</sup>   | 10.9 (0.4) <sup>B</sup>  | 10.2 (0.4) <sup>B</sup>  | 5.4 (0.4) <sup>C</sup>   | ***                             |
| <b>Maximum Temperature</b> |                          |                           |                          |                          |                          |                                 |
| January                    | 23.4 (0.3) <sup>A</sup>  | 23.2 (0.3) <sup>A</sup>   | 22 (0.3) <sup>A</sup>    | 17.3 (0.3) <sup>B</sup>  | 15 (0.4) <sup>C</sup>    | ***                             |
| February                   | 24.2 (0.3) <sup>AB</sup> | 24.3 (0.3) <sup>A</sup>   | 22.7 (0.3) <sup>B</sup>  | 18.6 (0.4) <sup>C</sup>  | 17.6 (0.4) <sup>C</sup>  | ***                             |
| March                      | 26.5 (0.2) <sup>A</sup>  | 26.8 (0.2) <sup>A</sup>   | 25.7 (0.3) <sup>A</sup>  | 21.6 (0.3) <sup>B</sup>  | 22 (0.3) <sup>B</sup>    | ***                             |
| April                      | 28.9 (0.2) <sup>A</sup>  | 29.4 (0.2) <sup>A</sup>   | 28.8 (0.2) <sup>A</sup>  | 24.7 (0.2) <sup>C</sup>  | 25.9 (0.3) <sup>B</sup>  | ***                             |
| May                        | 31.1 (0.1) <sup>B</sup>  | 32.3 (0.1) <sup>A</sup>   | 31.4 (0.1) <sup>B</sup>  | 27.7 (0.1) <sup>D</sup>  | 29.2 (0.2) <sup>C</sup>  | ***                             |
| June                       | 32.6 (0.1) <sup>C</sup>  | 33.7 (0.1) <sup>A</sup>   | 33 (0.1) <sup>BC</sup>   | 30.8 (0.1) <sup>D</sup>  | 33.5 (0.2) <sup>AB</sup> | ***                             |
| July                       | 32.6 (0.1) <sup>B</sup>  | 33.2 (0.1) <sup>AB</sup>  | 33 (0.1) <sup>AB</sup>   | 31.4 (0.1) <sup>C</sup>  | 33.4 (0.1) <sup>A</sup>  | ***                             |
| August                     | 32.8 (0.1) <sup>B</sup>  | 33.5 (0.1) <sup>AB</sup>  | 33.1 (0.1) <sup>AB</sup> | 31.6 (0.1) <sup>C</sup>  | 33.3 (0.1) <sup>A</sup>  | ***                             |
| September                  | 32.4 (0.1) <sup>AB</sup> | 32.9 (0.1) <sup>A</sup>   | 32.3 (0.1) <sup>B</sup>  | 29.8 (0.1) <sup>D</sup>  | 30.5 (0.2) <sup>C</sup>  | ***                             |
| October                    | 30.2 (0.2) <sup>AB</sup> | 30.5 (0.2) <sup>A</sup>   | 29.4 (0.1) <sup>B</sup>  | 26.6 (0.2) <sup>C</sup>  | 25.9 (0.3) <sup>C</sup>  | ***                             |
| November                   | 27 (0.2) <sup>A</sup>    | 27.1 (0.2) <sup>A</sup>   | 25.6 (0.2) <sup>B</sup>  | 21.8 (0.2) <sup>C</sup>  | 21.1 (0.3) <sup>C</sup>  | ***                             |
| December                   | 24.6 (0.3) <sup>AB</sup> | 24.8 (0.3) <sup>A</sup>   | 23.3 (0.3) <sup>B</sup>  | 19.3 (0.3) <sup>C</sup>  | 17.9 (0.4) <sup>D</sup>  | ***                             |

<sup>a</sup> $P>0.05$ , \*\*\* $P<0.001$ .

**Table 5.** Maximum number of consecutive days, maximum number of runs of 2 or more consecutive days and total number of days with minimum daily temperature  $\leq 0^{\circ}\text{C}$  in December, January and February during a five year period (2007-2011) for the sampling localities of *Anolis sagrei* and *Anolis carolinensis*. Data obtained from the United States National Oceanographic and Atmospheric Administration, Asheville, North Carolina. *A. sagrei* was not sampled at Punta Gorda, FL; *A. carolinensis* was not sampled at Naples, FL.

|                                                                                                        | Naples, FL | Punta Gorda, FL | Plant City, FL | St. Augustine, FL | Savannah, GA |
|--------------------------------------------------------------------------------------------------------|------------|-----------------|----------------|-------------------|--------------|
| Maximum number of consecutive days with minimum temperature $\leq 0^{\circ}\text{C}$                   | 1          | 3               | 5              | 5                 | 14           |
| Maximum number of runs of 2 or more consecutive days with minimum temperature $\leq 0^{\circ}\text{C}$ | 0          | 4               | 8              | 9                 | 31           |
| Total number of days with minimum temperature $\leq 0^{\circ}\text{C}$                                 | 1          | 16              | 29             | 35                | 125          |

**Table 6.** Pearson's correlations between the climatic variables, Field Critical Thermal Minima (CTMin) and Body Condition Index (BCI) for *Anolis sagrei* and *Anolis carolinensis*.

| Climatic Variables | Field CTMin             |                         |                         |                         | BCI                    |                        |                         |                       |
|--------------------|-------------------------|-------------------------|-------------------------|-------------------------|------------------------|------------------------|-------------------------|-----------------------|
|                    | <i>A. sagrei</i>        |                         | <i>A. carolinensis</i>  |                         | <i>A. sagrei</i>       |                        | <i>A. carolinensis</i>  |                       |
|                    | F                       | M                       | F                       | M                       | F                      | M                      | F                       | M                     |
| MMNT               | r = 0.56,<br>p = 0.001  | r = 0.69,<br>p = 0.001  | r = 0.40,<br>p = 0.001  | r = 0.39,<br>p = 0.001  | r = -0.29,<br>p = 0.03 | r = -0.26,<br>p = 0.07 | r = -0.37,<br>p = 0.004 | r = -0.5,<br>p<0.001  |
| EMNT               | r = 0.56,<br>p = 0.001  | r = 0.71,<br>p = 0.001  | r = 0.35,<br>p = 0.001  | r = 0.38,<br>p = 0.001  | r = -0.34,<br>p = 0.01 | r = -0.37,<br>p = 0.01 | r = -0.39,<br>p<0.002   | r = -0.52,<br>p<0.001 |
| DT0°               | r = -0.57,<br>p = 0.001 | r = -0.68,<br>p = 0.001 | r = -0.40,<br>p = 0.001 | r = -0.40,<br>p = 0.001 | r = 0.29,<br>p = 0.04  | r = 0.25,<br>p = 0.09  | r = 0.37,<br>p<0.003    | r = 0.51,<br>p<0.001  |
| TPCP               | r = -0.002,<br>p = 0.98 | r = -0.21,<br>p = 0.15  | r = 0.22,<br>p = 0.001  | r = 0.21,<br>p = 0.001  | r = 0.2,<br>p = 0.17   | r = 0.48,<br>p < 0.001 | r = -0.22,<br>p = 0.08  | r = -0.24,<br>p=0.07  |

**Table 7.** Mean daily minimum temperature and Total monthly precipitation for collection sites of *Anolis sagrei* and *Anolis carolinensis* for June and July of 2011. Mean monthly temperature, mean monthly minimum temperature, extreme minimum temperature and Number of days with temperature  $\leq 0^{\circ}\text{C}$  for the sites for the month of January between 2007 and 2011. Data obtained from the United States National Oceanographic and Atmospheric Administration, Asheville, North Carolina.

| Site              | June-July 2011                 |                                  | January 2007-2011             |                                  |                                 |                                                               |
|-------------------|--------------------------------|----------------------------------|-------------------------------|----------------------------------|---------------------------------|---------------------------------------------------------------|
|                   | Mean daily minimum Temperature | Total monthly Precipitation (mm) | Mean monthly temperature      | Mean monthly minimum temperature | Extreme minimum temperature     | Mean number of days with temperature $\leq 0^{\circ}\text{C}$ |
| Naples, FL        | 23.6 $\pm$ 0.1<br>(20.6-26.1)  | 332                              | 18.1 $\pm$ 0.9<br>(15.5-20.6) | 12.7 $\pm$ 0.9<br>(10.3-15.4)    | 1.8 $\pm$ 0.8<br>(0-4.4)        | 0.2 $\pm$ 0.2<br>(0-1)                                        |
| Punta Gorda, FL   | 22.5 $\pm$ 0.2<br>(17.8-25.6)  | 600.7                            | 16.9 $\pm$ 0.9<br>(14.3-19.5) | 10.3 $\pm$ 0.9<br>(7.9-13.5)     | -1.3 $\pm$ 0.6<br>(-3.3-0)      | 1.8 $\pm$ 0.8<br>(1-5)                                        |
| Plant City, FL    | 22.7 $\pm$ 0.1<br>(19.4-25.6)  | 829.6                            | 15.8 $\pm$ 0.9<br>(12.9-18)   | 9.5 $\pm$ 1<br>(6.6-12.4)        | -2.3 $\pm$ 1<br>(-5-1.1)        | 3.4 $\pm$ 1.4<br>(0-8)                                        |
| St. Augustine, FL | 23.5 $\pm$ 0.1<br>(21.1-26.1)  | 576.6                            | 12.6 $\pm$ 1<br>(10.2-15.3)   | 7.8 $\pm$ 1<br>(5.1-10.9)        | -2.2 $\pm$ 0.7<br>(-3.3-2.8)    | 5 $\pm$ 2<br>(7-17)                                           |
| Savannah, GA      | 22.7 $\pm$ 0.2<br>(17.8-27.8)  | 423.9                            | 9.2 $\pm$ 0.8<br>(7.3-11.4)   | 3.3 $\pm$ 0.9<br>(0.9-5.6)       | -5.8 $\pm$ 0.7<br>(-7.8-(-3.9)) | 10.4 $\pm$ 1.7<br>(9-17)                                      |

Data are expressed as mean  $\pm$  SE and range in parentheses.

## **Vita**

Laura Rubio-Rocha was born in Cali, Colombia, on August 13, 1986 to Vivian Marlene Rocha and Efraín Alfonso Rubio. She graduated from High School in Cali Colombia in 2003. She attended Universidad del Valle in Cali and received a B. S. in Biology in 2009, where she conducted research on the reproductive phenology and demography of a high altitude anole, *Anolis mariarum*. After graduation she moved to Medellín and spent six months working as research assistant conducting ecological and behavioral studies of a migratory bird, the cerulean warbler (*Setophaga cerulea*), in coffee plantations of the northern Andes of Colombia. Subsequently, she spent several months teaching high school biology in a bilingual school before enrolling in 2010 at The University of Tennessee, where she obtained her M. S. in Ecology and Evolutionary Biology in 2012.