Ontogeny of sleeping behaviour in four solitary species of *Anolis* lizard

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Abstract

Despite the nearly ubiquitous nature of sleep in the animal kingdom, behavioural research on sleep has focused on a few model organisms and widespread behaviours studied in artificial laboratory settings. In this study, we examine the ontogeny of sleeping perch selection in four closely related species of arboreal lizard in situ. The study species each occupy similar habitats and share 'solitary' ecology wherein potential lizard competitors are absent, thus providing four partially independent replicates with which to test hypotheses. We find that structural niche, analysed as perch height, varies by age and sex. Males sleep higher than females and adults sleep higher than juveniles in three of four species. Adults exhibit greater variability in sleep site selection. These patterns may reflect diurnal behaviour or indicate selective differences in sleeping perch variability across sex and ontogeny.

Keywords

Anolis, ontogeny, reptiles, sleep, squamata.

1. Introduction

Ontogenetic changes in sleeping behaviour and physiology have been observed in a phylogenetically wide range of taxa. Longer periods of sleep in early ontogeny have been observed in mammals, birds, fish, turtles, fruit flies and roundworms (Roffwarg et al., 1966; Szymczak, 1987; Shaw et al., 2000; Raizen et al., 2008; Steinmeyer et al, 2010; Hasan et al., 2012; Sorribes et al., 2013; Hart et al., 2016; Kayser & Biron, 2016; Dilley et al., 2018; Burger et al., 2020). In mammals and birds, proportion of time in Rapid Eye Movement

(REM) sleep is highest in new-borns (Roffwarg et al., 1966; Jouvet-Mounier et al., 1969; Frank & Heller, 1997; Thurber et al., 2008; Scriba et al., 2013). In addition to physiological changes over ontogeny, Dilley et al. (2018) found that adult *Drosophila* show sleeping site fidelity whereas juvenile site selection was more variable. The shared presence of longer sleep in juveniles relative to adults across the animal kingdom suggests the potential for deep phylogenetic conservation of sleeping behaviours.

Sleep in non-avian reptiles (i.e., crocodilians, turtles, squamates) has been understudied relative to birds and mammals, especially with reference to behavioural and ontogenetic variation (reviewed in Libourel & Herrel, 2016; Mohanty et al., 2021). Physiological aspects of sleep (i.e., REM, Non-REM cycles) potentially share homology suitable for comparative study across vast evolutionary scales (Corner, 1977; Shein-Idelson et al., 2016; but see Libourel & Barrillot, 2020). Ontogenetic behavioural traits beget novel challenges for evolutionary comparison. In particular, the lack of post-hatching parental care in most species subjects juvenile turtles and lepidosaurs to predation and food procurement pressures not experienced by young archosaurs or mammals that undergo parental care. Thus lizards and turtles pose a distinctive model system for observing behavioural ontogeny as it pertains to sleep relative to the more studied mammal and bird clades.

Juveniles of a species frequently occupy a different niche than adults (Werner & Gilliam, 1984). Many aspects of an organism's fitness such as preferred diet, potential predation, and interspecific competition for resources such as territories each relate to size and by extension ontogeny. Sleep site selection presents a behavioural response to these variables when in a state of reduced awareness (Lima et al., 2005). The changing selective pressures over ontogeny set the expectation that behavioural responses also will vary. This principle is demonstrated in juvenile sea turtles (*Chelonia mydas*) foregoing increased restfulness of deeper waters and proximity to foraging grounds for the safety of the shallows (Hart et al., 2016). Similarly juvenile Galapagos Land Iguanas (*Conolophus subcristatus*), which are susceptible to avian predation, invariably sleep in concealed crevices. Larger adults, without similar vulnerability, frequently sleep at more exposed sites allowing greater ability to thermoregulate (Christian et al., 1984).

Behavioural studies of sleep commonly are conducted in laboratory settings, often without regard to natural light and heat cycles (e.g., Flanigan 1973; Ayala-Guerrero & Mexicano, 2008; Aulsebrook et al., 2016; Russart &

Nelson, 2018). Such studies are valuable as controlled experiments but may generate artefactual captive behaviours and/or fail to account for significant ecological factors in nature that may affect sleep behaviour (e.g., exposure, predation, food availability, intra- and interspecific interactions; Anderson, 1998; Ramakrishnan & Coss, 2001; Lima et al., 2005; Calisi & Bentley, 2009). Field studies may corroborate behaviours observed in laboratory environments and contextualize patterns within the ecological framework that dictates the organism's fitness (see Rattenborg et al., 2017; Mohanty et al., 2021).

The lizard genus *Anolis* (anoles) provides an apt system to study reptilian sleep in a field environment. Widespread throughout the neotropics, anoles are present on nearly every vegetated land mass in the Caribbean (Powell & Henderson, 2012). On many islands, anoles are unaccompanied by congeners and other ecologically similar lizards, allowing for replicable experimentation and observation of the behavioural patterns of single species unfettered by interspecific competition. Caribbean anoles can occur at high population densities, approaching one per square metre (Schoener & Schoener, 1980). Finding sleeping anoles poses little difficulty, as they can be observed at night contrasting with leaves or branches. A wealth of diurnal studies have been conducted on anoles, resulting in possibly unparalleled understanding among nonhuman vertebrates of ecological factors that affect diurnal behaviours in these organisms (see Losos, 2009). Chief among these factors is interspecific competition between congeners leading to altered behaviour (e.g., Schoener, 1968; Losos, 1994). Relative to daytime anole research, few studies have examined anole sleeping patterns (but see, e.g., Clark & Gillingham, 1990; Poche et al., 2005; Singhal et al., 2007; Storks & Leal, 2020 for detailed observations). These studies have demonstrated variation in perch height and perch substrate within and between species. No study has examined hypothesized or observed patterns in the absence of congeners or in replicated studies across comparable sites.

Here we test for ontogenetic change in sleeping behaviour in four ecologically, morphologically, and phylogenetically similar lizard species that inhabit similar environments. We test for homogeneity of variance and similarity of central tendencies in adult males, females, and juveniles as a proximate estimate of structural niche breadth. Previous research compared diurnal structural niche in anoles between island systems with and without anoline congeners, finding isolated species occupy a broader structural niche

(Rand & Rand, 1967; Lister, 1976). Extrapolating from demonstrated niche expansions in solitary species, we hypothesize that each of the four species will sleep at similar heights stemming from the absence of interspecific competition. We further hypothesize that in each species, adults will sleep higher than juveniles with greater variance and adult males will sleep higher than adult females due to selective superiority of higher perches.

2. Materials and methods

2.1. Data collection

We collected data on four species of *Anolis*: *A. conspersus* ($N = 319$ observations) from Grand Cayman Island, *A. luteosignifer* ($N = 176$) from Cayman Brac, *A. marmoratus* ($N = 241$) from Guadeloupe, and *A. ferreus* ($N = 31$) from Marie-Galante. Our study incorporated 17 sites over the four islands (Figures 1–3). Study sites were selected by analysing satellite imagery for habitat conducive to harbouring arboreal lizards (i.e., forested areas), or areas convenient for sampling (i.e., the grounds of our residence during the study). We characterized these sites as either developed or undeveloped. Our characterization of undeveloped habitat indicates the habitat was connected to a preserve or forest and was unlikely to have been adulterated. Developed habitats include grounds of residences and groves of trees isolated from large preserves. All coordinates presented use Datum WGS 1984, with sites listed from west to east.

   Lizards were located at night between the hours of 1900 and 0700, captured, sexed, and measured for snout-vent length (SVL). Nighttime visual encounter surveys were conducted along suitable habitat, following roads and trails as applicable. In open areas not defined by a linear pathway, each researcher moved through a designated area of habitat searching for sleeping lizards. Perch height was recorded according to convention as distance above the ground at which the lizard was observed (Rand, 1964). The search was conducted from the ground to the forest canopy. We note each observed lizard was temporarily removed from the habitat in accordance with IACUC protocol 19-200091-MC to avoid pseudo replication on consecutive nights. All lizards were returned to their respective localities unharmed following the study.

   Individual lizards were categorized by examination for sexually dimorphic traits (e.g., hemipenes and size or presence of dewlap). As juvenile
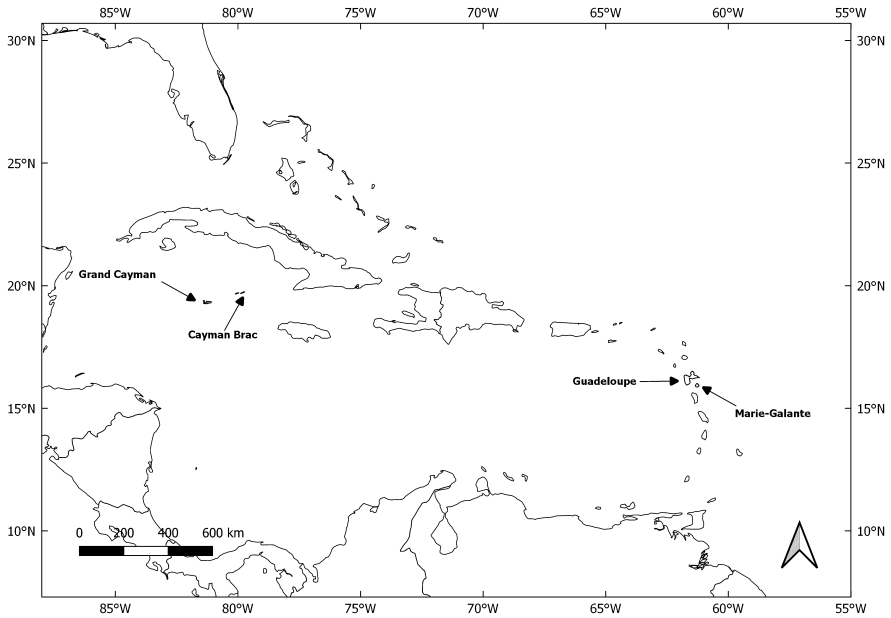
Figure 1. Map of the Caribbean with sampled islands indicated.

anoles are not sexually dimorphic until maturity and often resemble small adult females until mature, we followed the approaches of previous studies detailing the approximate size of sexual maturity.

2.1.1. Grand Cayman

We selected four sites to study *A. conspersus* on Grand Cayman Island (Figure 2). This species is considered a trunk-crown ecomorph, closely related to the Jamaican *A. grahami* (Losos, 2009). As such, it is expected *a priori* to be a primarily arboreal form. Data collection took place over the four nights of October 6–8 and 11, 2018. We followed the methods of Schoener (1967) in categorizing all *A. conspersus* below 34 mm snout–vent length (SVL) as juveniles. All sites were visited for approximately two hours each night. Of the four sites, we characterized three as undeveloped and one as developed. The three undeveloped sites (Mastic Trail 19.3136° N, 81.1913° W; Road near Queen Elizabeth II Biological Preserve 19.3177° N, 81.169° W; Road to Collier's Wilderness Reserve 19.328°N, 81.086°W) consisted of heavily forested areas with abundant underbrush, providing viable perches from the ground to the tops of trees. Sampling was conducted primarily along pathways conducive to walking (i.e., roads, trails). The developed site (Residence
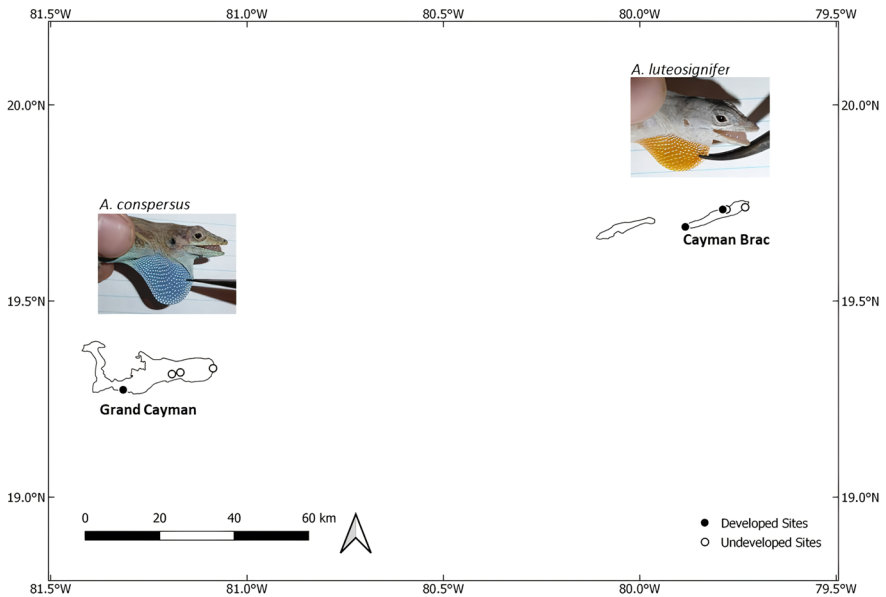
Figure 2. Study sites in Grand Cayman and Cayman Brac Islands. Solid dots indicate undisturbed habitat, open circles indicate developed areas.

at intersection of Jasmine Lane, Shamrock Road 19.273° N, 81.3154° W) had mature trees, but underbrush was cleared on residential grounds to a greater extent than the undeveloped sites.

2.1.2. Cayman Brac

We selected four sites to study *A. luteosignifer* on Cayman Brac (Figure 2). Data collection took place over the two nights of October 9th and 10th, 2018. Losos & DeQuieroz (1997) classify this species as a trunk-ground anole based on ecological data, indicating a low degree of arboreality relative to congeners. Given the dearth of body size data on *A. luteosignifer*, we refer to Delaney & Warner's (2016) cut-off for the closely related *A. sagrei* at 34 mm. Notably, as recently as 2017, *A. sagrei* and *A. luteosignifer* were considered conspecific (Kolbe et al., 2017). We note that the treatment of *A. luteosignifer* as distinct from *A. sagrei* appears to vary by author, although the majority favour separate species status for these forms (see Oppel et al., 2019; Prado-Irwin et al., 2019; Hedges et al., 2020). As on Grand Cayman, we sampled each site on both nights. We characterized two sites (Bluff dead end near Glennie Dilbert Road 19.733° N, 79.779° W; near Great Cave
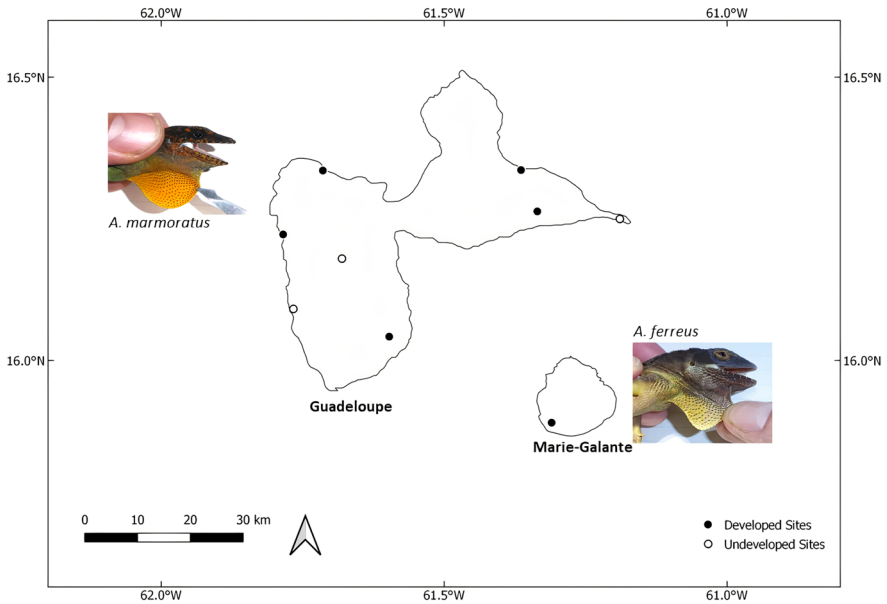
Figure 3. Study sites in Guadeloupe and Marie-Galante. Solid dots indicate undisturbed habitat, open circles indicate developed areas.

19.739° N, 79.732° W) as undeveloped. These sites consisted of xeric scrub and mature forests characteristic of the island. We sampled at two developed sites: West End Community Park (19.689° N, 79.884° W) and the grounds of Featherstone Cottage (19.740° N, 79.789° W). The community park grounds had mature trees, but cleared underbrush. We sampled both the interior core of the grounds as well as the edge of the surrounding wooded area. The edges of the park resembled undeveloped habitat with plentiful perches at all observable heights. The grounds of Featherstone Cottage were similarly cleared, and characterized by beach shrubs up to 2 m. The edges of the grounds were undeveloped with potential perches at all observable heights.

2.1.3. Guadeloupe

We sampled *A. marmoratus* at eight sites around Guadeloupe. Data collection occurred over the four nights of March 11–13 and 15, 2018. We followed the methods of Lazell (1972) to consider *A. marmoratus* as juvenile if SVL measured less than 48 mm. Losos & DeQuieroz (1997) classify this species as a trunk crown anole based on ecological and morphological evidence. Of our eight sites, we categorized three as undeveloped (along N2, 5 kilometres

south of Bouillante 16.091° N, 61.766° W; off D23 west of Vernou 16.179° N, 61.680° W; Along D118 16.250° N, 61.189° W). These sites were characterized by mature broadleaf forests with viable perches at all observable heights. Of the five developed sites (Grove of trees along N2 west of Sainte Rose 16.222° N, 61.784° W; small forest west of Sainte Rose 16.335° N, 61.714° W; grounds of residence west of Capestre Belle Eau 16.042° N, 64.597° W; grove of trees west of Le Moule 16.336° N, 61.364° W; grounds of residence south of Bragelogne 16.263° N, 61.335° W), all were a mix of cleared to uncleared landscape with mature trees, harbouring viable perches at all observable heights.

2.1.4. Marie-Galante

We sampled *A. ferreus* over much of Marie-Galante on the night of March 14th, 2018. Although this species has not been found to conform strongly to any described ecomorph, Losos & DeQuieroz (1997) considered this species to be closer to trunk-crown than to any other ecomorph or to a 'generalist'. We followed the approaches of Lazell (1972) to consider *A. ferreus* as a juvenile for individuals with SVL less than 48 mm. We characterized our site outside of Grand-Bourg (15.891° N, 61.312° W) as developed due to proximity to residential areas, but note this area harboured some mature tropical broadleaf forest.

2.2. Statistical analyses

We measured perch height as the primary indicator of habitat use. The data were divided by species, age, and sex. All statistics were done in R (version 4.0.2; R Core Team, 2020). Each species was evaluated for normally distributed residuals with the Anderson-Darling Test under the R package 'nortest' (Gross & Ligges, 2015). As none of the species met the assumption of normality ($p < 0.0001$), the Kruskal–Wallis H-test was used to determine equivalency of the median perch height of adult males, adult females, and juveniles. Dunn's Multiple Comparisons Test was used post-hoc to analyse pairwise differences in the medians between the three data classes (species, age, sex). p values from Dunn's Multiple Comparisons Test were adjusted using the Holm–Bonferroni method. The Kruskal-Wallis test and Dunn's post-hoc were calculated with R package 'FSA' (Ogle et al., 2021).

Differences between perch heights in developed and undeveloped sites were evaluated at the class level within each species (e.g., adult male

A. luteosignifer in developed compared to undeveloped) using the Mann–Whitney *U*-test. *Anolis ferreus* was not tested in this manner as data was only collected at one site.

Homoscedasticity, as a representation of structural niche breadth, was analysed with the Fligner–Killeen Test of Homogeneity of Variances under the R package 'car' (Fox & Weisberg, 2019). To our knowledge, no post-hoc test exists to examine pairwise differences in a three-sample test of homoscedasticity. As such, we applied Fligner–Killeen pairwise to males–females, males–juveniles and females–juveniles within species.

We examined the overall hypotheses of difference in perch height and perch variance between classes using Brown's (1975) extension of Fisher's combined probabilities test, combining *p*-values for each of the four species. We incorporated a correlation matrix of phylogenetic branch length under Brownian motion from the most recent tree (Poe et al., 2017), allow-ing for the correction of non-independence of species (i.e., closely related species share more evolutionary history potentially resulting in conserved behavioural traits; Felsenstein 1985). Statistics were calculated under R package 'poolr' (Cinar & Viechtbauer, 2021) and checked manually.

In addition to perch height, we collected data on perch type coded dichoto-mously as twig vs leaf and analysed by species using Pearson's chi-squared test under the R package 'vcd' (Meyer et al., 2020). Sex and age classes were examined by evaluating standardized (Pearson's) residuals, deemed signifi-cant when the absolute value of the residual exceeds two.

3. Results

Here we report central tendencies of perch height for the four species included in this study (Table 1, Figure 4)

In our surveys of perch height usage in solitary island anoles, we found significant differences between age and sex within three of four species (Kruskal–Wallis, *A. conspersus* $p = 3.1e-13$; *A. marmoratus* $p = 6.5e-5$; *A. ferreus* $p = 0.036$). In these three species, adult lizards slept on higher perches, but notably were not absent from lower perches. Juvenile lizards exhibited greater habitat specificity, occupying lower perches in greater abundance. Within these species, Dunn's Multiple Comparisons Test found support for the consistent difference between males and juvenile, while females appeared to represent an intermediate value statistically indistinct

Table 1.
Central tendencies by *Anolis* species and class.

|	Mean (m)	Median (m)	Range (m)	Variance (m^2)	Interquartile range (m)
A. conspersus males ($n = 106$)	2.6	2.1	0.2–6.5	1.8	1.7–3.0
A. conspersus females ($n = 128$)	2.0	1.7	0.3–6.0	1.3	1.2–2.4
A. conspersus juveniles ($n = 85$)	1.4	1.1	0.2–4.0	0.6	1.0–1.9
A. luteosignifer males ($n = 34$)	1.3	1.0	0.1–4.6	1.45	0.5–1.5
A. luteosignifer females ($n = 45$)	1.4	1.1	0.2–6.0	1.3	1.0–1.5
A. luteosignifer juveniles ($n = 97$)	1.3	1.1	0.1–4.5	0.8	0.5–1.7
A. marmoratus males ($n = 92$)	2.0	1.8	0.3–7.0	1.3	1.4–2.3
A. marmoratus females ($n = 35$)	1.6	1.5	0.5–5.0	0.6	1.0–1.8
A. marmoratus juveniles ($n = 113$)	1.5	1.4	0.1–6.0	0.8	1.0–1.9
A. ferreus males ($n = 15$)	2.8	2.4	1.0–7.0	2.9	1.7–3.5
A. ferreus females ($n = 5$)	1.8	1.8	1.0–3.0	0.7	1.0–2.1
A. ferreus juveniles ($n = 11$)	1.4	1.2	0.7–2.6	0.36	1.1–1.7
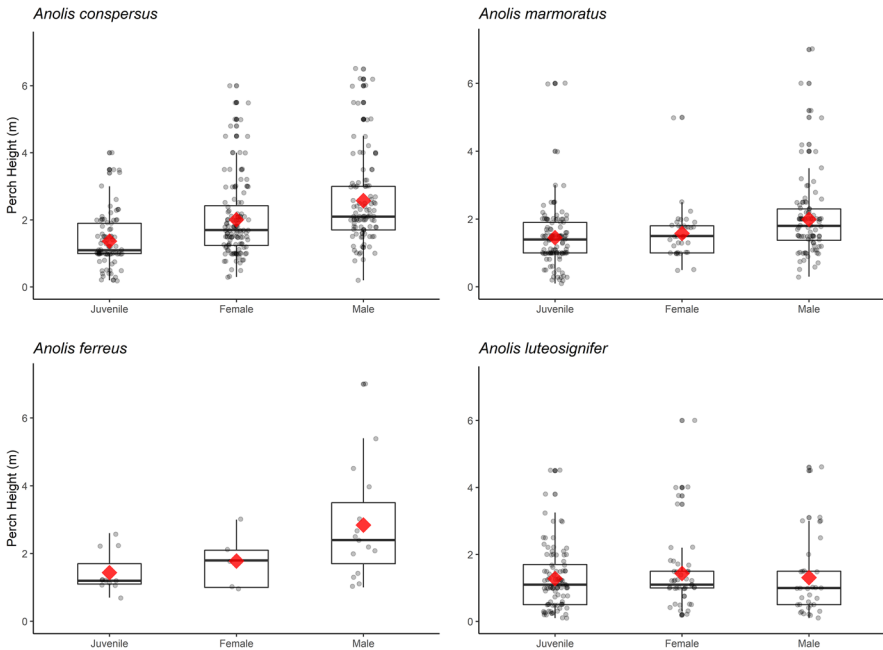
Figure 4. Perch height for age and sex of each species. Mean is indicated by the red diamond.

Table 2.

Pairwise comparisons of perch height between males, females and juveniles of each species using Dunn's Multiple Comparisons Test.

Species	Comparison	p value
A. conspersus	Male–Female	1.1e−04*
A. conspersus	Male–Juvenile	9.8e−14*
A. conspersus	Female–Juvenile	4.2e−05*
A. luteosignifer	Male–Female	0.69
A. luteosignifer	Male–Juvenile	0.76
A. luteosignifer	Female–Juvenile	0.58
A. marmoratus	Male–Female	0.064
A. marmoratus	Male–Juvenile	3.8e−05*
A. marmoratus	Female–Juvenile	0.33
A. ferreus	Male–Female	0.24
A. ferreus	Male–Juvenile	0.043*
A. ferreus	Female–Juvenile	0.76

Significant results ($p < 0.05$) are indicated with an asterisk and underlining across the row. Significance equates to a statistical difference in the median.

from either the males or the juveniles in two species (Table 2). Combining adult males and females into a single category to reveal ontogenetic separation provided a similar result. In this combined test of ontogenetic separation, support was particularly strong in *A. conspersus* and *A. marmoratus* ($p < 0.0005$). In *A. luteosignifer*, there was no support for the idea that sex or age class influences perch height (Kruskal–Wallis, $p = 0.48$). Similarly, pair-wise comparisons do not support any differentiation by class (Table 2).

Using Brown's method to test the overall hypothesis of classes (species, age, sex) varying in sleep height, we found strong evidence indicating a difference in median perch heights between classes. As may be expected from the specific comparisons, we found strong support for differential habitat use between adult males and juveniles ($p = 3.8e−10$). Adult females, when combined, had a significantly different median from juveniles ($p = 0.0098$) and males ($p = 0.0049$).

Evaluating the difference in developed and undeveloped sites, we found *A. conspersus* females and juveniles both slept significantly higher in developed sites (Mann–Whitney U-test; *A. conspersus* females $p < 0.001$; *A. conspersus* juveniles $p = 0.001$). The impact of development did not have a statistically appreciable effect ($p > 0.05$) on males of *A. conspersus* nor on any class of *A. luteosignifer* or *A. marmoratus*.

In two of four species (*A. conspersus*, *A. ferreus*) we found a significant difference in variance between males and juveniles, with males exhibiting greater variance than juveniles (Table 3). Adult females of these species were statistically indistinguishable from adult males and juveniles. Neither *A. luteosignifer* nor *A. marmoratus* showed appreciable differences between any of the classes. When comparing homoscedasticity between combined adults (i.e., regardless of sex) and juveniles, we found support for differences in variance in the two species distinguishable by class (*A. conspersus*, *A. ferreus*) but failed to recover a significant result in *A. marmoratus* or *A. luteosignifer*. As with the 'by-class' test, adults exhibited greater variance than juveniles.

Applying again Fisher's combined probability test to assess the hypothesis of differing variance between classes, we found strong support for distinct variance between adult males and juveniles ($p = 0.013$). We did not find significant support for differences between adult males and females ($p = 0.16$), nor females and juveniles ($p = 0.15$), suggesting the variance of females occupies a statistically indistinct intermediate state between the other classes. In examining microhabitat by perch type, we did not find strong association between classes and sleeping perch. In *A. luteosignifer*, *A. marmoratus*, and *A. ferreus* preferences between twigs and leaves are statistically nonsignifi-

Table 3.
Pairwise comparisons of homoscedasticity of perch heights using Fligner–Killeen.

Species	Comparison	p value
A. conspersus	Male–Female	0.27
A. conspersus	Male–Juvenile	0.0038*
A. conspersus	Female–Juvenile	0.039*
A. luteosignifer	Male–Female	0.34
A. luteosignifer	Male–Juvenile	0.37
A. luteosignifer	Female–Juvenile	0.50
A. marmoratus	Male–Female	0.063
A. marmoratus	Male–Juvenile	0.20
A. marmoratus	Female–Juvenile	0.14
A. ferreus	Male–Female	0.30
A. ferreus	Male–Juvenile	0.026*
A. ferreus	Female–Juvenile	0.50

Significant p values ($p < 0.05$) indicate heterogeneity of variance and are indicated by an asterisk and underlining across the row. Significance indicates heterogeneity between the compared variances.

cant in males, females, and juveniles ($p = 0.281$–0.410). Our data supported an association of *A. conspersus* with leaves ($p < 0.005$), however this association between *A. conspersus* preferentially sleeping on leaves was supported solely by females of the species ($R = 2.09$). Neither adult males nor juveniles exhibited a preference for either substrate ($R = -0.134$–0.124). Previous research (Singhal et al., 2007) has shown that substrate use at night differs from diurnal use, with individuals sleeping on leaves they would not occupy during the day. To our knowledge, the effect of age or sex of the individual on sleeping perch substrate has not been examined. We are unable to concoct a believable hypothesis for why only females of *A. conspersus*, which share varying degrees of sex/age/ecology/species status with the other 11 studied classes, would prefer leaves. Therefore we will not discuss these results further.

4. Discussion

Here we have shown variation among age and sex classes within species of ecologically similar neotropical lizards. Statistical analyses of central tendencies indicate that in three of four species males sleep higher than females and adults sleep higher than juveniles (Figure 2, Table 2). When the four species are treated as semi-independent experimental replicates, Fisher's combined probabilities test strongly supports interclass differences in perch height median but mixed support for heterogeneity of variance. The impact of human development appears to have a negligible effect on the overall trends. Although females and juveniles of *A. conspersus* were found to perch significantly higher in disturbed versus undisturbed areas, this difference did not result in reduced distinction between the classes in this species. We hypothesize this is an artifact of the removal of underbrush in the disturbed site. With low perches removed, the individuals typically found in these perches moved higher up to the lowest available perches.

We comment first on plausible explanations for these patterns and discuss potential implications for *A. luteosignifer* deviating from recovered trends. Finally, we discuss the broader evolutionary implications of our results.

4.1. Adults sleep in different perch ranges than juveniles

Adults were found to sleep in different strata and with greater variance than juveniles in three of four replicates. Ontogenetic shifts in habitat use

have been recorded for diurnal behaviours in anoles (Stamps, 1983), and our results may reflect an effect of diurnal behaviour on nocturnal sleep site. Previous studies have suggested the possibility of correlation between diurnal activity and nocturnal sleep sites in arboreal lizards (Singhal et al., 2007; Cabrera-Guzman & Reynoso, 2010; McCranie & Kohler, 2015). Avery (1988) notes the predominance of juvenile *A. conspersus* on or near the ground, juxtaposed against adult lizards that were most often found on arboreal perches. His observations during the day mirror our sleep observations for *A. conspersus*, noting that adult males occupy higher perches than adult females.

This pattern of males perching higher than females is observed in several species of anole (e.g., *A. acutus* (Ruibal & Philobosan, 1974); *A. sagrei* (Lister, 1976); *A. gundlachi* (Irschick et al., 2000); *A. lineatopus* (Irschick et al., 2000), and others) and may be related to competitive superiority of higher perch sites during the day. For example, the higher perching of males may be expected in the context of diurnal territorial behaviour. That is, perching higher may allow dominant males (Stamps, 1973; Chandler & Tolson, 1990) to visually communicate to females and rivals over a greater distance. Alternative competitive diurnal explanations for higher perching in males include the potential superiority of higher perch heights for physiological reasons such as greater access to favourable thermoregulatory sites.

Distinct from diurnal effects, there likely are nocturnal selection pressures that at least partially dictate anole sleeping perch (Anderson, 1998). When sleeping, lizards lose the ability to thermoregulate through behaviour. Thermal tendencies of potential sleep sites influence metabolic processes of the individual. As the environment cools overnight, sites near the ground remain warmer due to radiant heat from the earth (Geiger, 1965). Applying the principles of Bergmann's Rule, smaller lizards with a greater surface area to volume ratio are more susceptible to heat dissipation and the negative effects of cooling temperatures. In this way, it is expected that smaller lizards (i.e., juveniles) may require warmer and more stable temperatures near the ground. It has been observed that decreased temperatures (as a function of elevation) lead to decreased arboreality as a thermoregulatory mechanism in *Sceloporus* lizards (Adolph, 1990). This requirement may play a role in our results, wherein we found juveniles to sleep lower than adults and females, which are smaller than males in each of our study species, to sleep lower than males. Other scale-dependent explanations for these results such as arboreal

performance differences and body size related energetics also are possible (see Keren-Rotem et al., 2006; Mohanty et al., 2016).

Anolis lizards in assemblages of multiple congeners are a model system for studying niche partitioning as a mechanism to decrease competition for resources (e.g., food, thermal environment, safety; Rand, 1967; Schoener, 1968; Perez-Martinez et al., 2021). Our sleep results may indicate nocturnal manifestation of a diurnal effect (see above), or may indicate competitive partitioning of sleeping sites. It is possible that 'interclass' competition, similar to interspecific competition, may exclude juveniles from higher perches where they are not able to compete with the larger adults. Schoener (1967) demonstrated intraspecific differences in prey type by class in *A. conspersus* and Butler et al. (2000) demonstrated that anole sexes occupy different diurnal niches within species. These results suggest the possibility of a similar nocturnal ontogenetic phenomenon in our study. That is, ecological factors may exclude juveniles from sleeping on higher perches preferred by adults.

Predator avoidance may also pose another variable in sleep site selection. Sleep site selection has implications in avoiding both nocturnal predators and diurnal predators in the mornings when mobility is reduced as a function of cooler temperatures. This study considered perch height as the primary structural aspect, though other variables (perch width, distance to trunk) may serve an important role in predator avoidance (Mohanty et al., 2016; Bors et al., 2020). During our study, we observed nocturnally active arboreal snakes (*Cubophis caymenensis*) and Cuban Tree Frogs (*Osteopilus septentrionalis*) – both known anole predators (de Armas & Hernande-Peraza, 2002; Henderson & Powell, 2009).

The threat of predation from conspecific organisms (i.e., cannibalism) has been documented as a causal agent in ontogenetic partitioning in several clades of squamates including chameleons (Keren-Rotem, 2006) and varanids (Auffenburg, 1981). Intraspecific cannibalism with large males eating juveniles is a commonly reported phenomenon in Caribbean anoles and has been reported in our focal species (reviewed in Powell & Watkins, 2014). It is not unrealistic to hypothesize that the threat of predation may contribute to juveniles avoiding sleeping perches near large males.

4.2. The exception: Anolis luteosignifer

The results with *A. luteosignifer*, wherein there were no significant differences among classes in perch height, confound the above discussed pattern

of ontogenetic partitioning. *Anolis luteosignifer*, while sparsely studied, is similar morphologically, ecologically, and evolutionarily to well-studied *A. sagrei. Anolis sagrei* is well known for its low degree of arboreality compared with other *Anolis* lizards (e.g., Collette, 1961; Schoener, 1968; Lister, 1976). Our sleep results may simply reflect the narrower range of lower perches used diurnally by *A. luteosignifer* (i.e., *sagrei*). More arboreal counterparts *A. marmoratus*, *A. ferreus* and *A. conspersus* (Losos & deQueiroz, 1997) consistently display a trend upwards in sleep site over ontogeny. Complicating this interpretation are the results of Lister (1976), who found significantly higher diurnal perching in adults versus juveniles of *A. sagrei*. Our sleep results may reflect lower power in our study, or an intriguing case where sleeping behaviour does not reflect diurnal behaviour. Our current data are unable to distinguish these hypotheses.

4.3. Broader evolutionary comparisons

In laboratory experiments with fruit flies, adults exhibit sleep site specificity whereas juveniles display no discernible preference (Dilley et al., 2018). That is, adults sleep less variably than juveniles. Issues with individual versus population variance aside (Kamath & Losos, 2017), our study finds that *Anolis* lizards may display an opposite pattern, with adults of *A. conspersus* and *A. ferreus* exhibiting less specificity toward a particular region (i.e., greater variance) of available habitats while juveniles tend to occupy lower perches exclusively (Table 3). This trend is seen most strikingly in the adult male/juvenile comparison, with females inhabiting a median ground that may or may not be statistically distinct from either class. In *A. luteosignifer* and *A. marmoratus*, there is no significant difference in the variance of perch heights between adults and juveniles, regardless of sex.

Many studies of sleep behaviour are of a single species, with results from that species explicitly hypothesized to be generalizable far beyond the focal species (e.g., comparison of *Drosophila* vs. humans) and tacitly assumed to be present in close evolutionary relatives. For example, discussion of the potential homology of extended sleep in early ontogeny across vertebrates (e.g., Olofsson & de Bono, 2008; Dilley et al., 2018) is predicated on the assumption that conditions in *Drosophila melanogaster* are present in other *Drosophila*, other Dipterans, other Arthropods, and other invertebrates. Our results indicate ontogenetic patterns of sleeping behaviour are not consistent even between close relatives with similar ecologies (compare results of *A.*

luteosignifer to results from the other studied species). We suggest that cross-clade comparisons between distant relatives should be made with caution.

4.4. Summary

Here we have shown that sleep site selection varies by age and sex in three of four species of solitary *Anolis*. Overall trends include males sleeping higher than females, adults sleeping higher than juveniles, and males sleeping more variably than juveniles. These patterns may reflect diurnal habitat use or selective differences in sleeping perch related to ontogeny. Future work should attempt to tease apart these hypotheses and explain the exceptional behaviour of *A. luteosignifer* wherein, in contrast to diurnal patterns, no sex- or age- related trends in sleeping behaviour were evident.

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