

# Hurricane Irma induces divergent behavioral and hormonal impacts on an urban and forest population of invasive *Anolis* lizards: evidence for an urban resilience hypothesis

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Submitted: 9 June 2020; Received (in revised form): 21 September 2020. Accepted: 9 June 2020

## Abstract

Hurricanes can have both profound short-term effects on animal populations and serve as long-term drivers of evolutionary change. Animals inhabiting varying habitats may differ in their response to hurricane impacts. Increasing evidence suggests that animals from urban areas exhibit different behavioral and physiological traits compared to rural counterparts, including attenuated hormonal stress responses and a lowered propensity for flight behavior. A unique opportunity was presented when Hurricane Irma hit Florida on 10 September 2017 and interrupted a study of invasive brown anoles (*Anolis sagrei*) at an urban and a forest. Using data collected before and after Hurricane Irma, we documented that forest anoles exhibited a greater avoidance of people and more male territorial behavior for a longer period of time following the hurricane. Post-hurricane both populations increased corticosterone concentrations post-capture stress, but urban anoles recovered 2 weeks faster than forest conspecifics. A dexamethasone suppression experiment suggested that these population differences were the result of forest anoles having a less effective negative feedback regulating corticosterone secretion. In the brain, forest anoles had higher corticosterone concentrations within the amygdala and parts of the cortex associated with stress than urban lizards. One explanation may be Hurricane Irma brought flooding and debris that altered the landscape leading to behavioral instability, and urban lizards already exhibited ecological adjustments that permitted a more rapid recovery (i.e. the ‘urban resilience’ hypothesis). Testing if urban animals are more resilient to natural disasters can inform conservationists interested in understanding their role in facilitating invasive species expansion and what their increasing presence may indicate for animal populations.

**Key words:** stress, hurricanes, urbanization, resilience, aggression

## Introduction

Hurricanes can have significant and long-lasting impacts on animal communities, through direct depopulation (Losos, Schoener, and Spiller 2003; Schoener, Spiller, and Losos 2004; Feehan, Scheibling, and Lauzon-Guay 2012), shifts in species composition (Meshaka 1993) and altered habitat succession patterns and nutrient cycling (Ostertag, Scatena, and Silver 2003).

Hurricanes have dramatic consequences for conservation (Reagan 1991; Ostertag, Scatena, and Silver 2003; Feehan, Scheibling, and Lauzon-Guay 2012; Vandermeer 2017; Donihue et al. 2018) and understanding resilience in an urban ecology can inform animal invasions, which typically begin in cities (Shochat et al. 2010). A well-known example is the introduction of escaped Burmese pythons (*Python bivittatus*) following

Hurricane Andrew in 1992 (Meshaka 1993; Willson, Dorcas, and Snow 2011), which has decimated natural lands in South Florida. Climate change will exacerbate the frequency and severity of tropical storms including hurricanes, and determining if urban animals have greater resilience has important implications for monitoring animal populations in rapidly changing environments.

Much less studied are the physiological impacts of destructive weather disturbances, which may impose significant impacts on fitness (Donihue et al. 2018) and drive evolutionary processes (Donihue et al. 2020). A potential link between environmental conditions and physiology is the adrenal secretion of glucocorticoid (GC) steroid hormones, such as corticosterone (CORT) in reptiles. Most research on GCs has focused on their role in the hormonal response to stress, where they exert complex and multi-faceted short- and long-term effects on energy metabolism, the immune system, reproduction and behavior (Wingfield and Sapolsky 2003). One feature of GCs is their ability to adjust neurochemistry to alter future reactions to stressful stimuli (Rodrigues, LeDoux, and Sapolsky 2009; Karatsoreos and McEwen 2011), and this is a central mechanism in physiological resilience (Srinivasan, Shariff, and Bartlett 2013; Reul et al. 2015). Resilience is a complex multidimensional construct (Rodrigues, LeDoux, and Sapolsky 2009; Wingfield, Kelley, and Angelier 2011) describing the ability to successfully acclimate and rapidly recover from a severe challenge (Srinivasan, Shariff, and Bartlett 2013) and high resilience to regularly encountered challenges would be strongly favored by natural selection. On the contrary, low resilience may negatively impact survival or reproduction and thus impose fitness costs (Meylan, Miles, and Clobert 2012). Thus, studying the ability to effectively enact, regulate and then restrict the hormonal stress response can be a fruitful way to investigate physiological resilience in a natural setting.

The regulation of GC secretion involves a negative feedback loop, where systemic hormones interact with receptors within the brain (Myers, McKlveen, and Herman 2012; Herman 2013). Low circulating GC levels over time can up-regulate receptors in stress-associated brain regions (e.g. hypothalamus, hippocampus and amygdala) to increase their sensitivity and permit an effective negative feedback (Oitzl et al. 2010; Herman 2013). However, during prolonged stress, excessively high GC exposure in the brain may permanently deregulate the negative feedback, resulting in a disproportionate overreaction to a potential future stress exposure (Herman 2013). However, in each population, some individuals may be more resilient to GC exposure and thus maintain a robust negative feedback that permits recovery from stress (Conrad 2008; Herman 2013). Despite some experimental research with traditional model species the relationship between circulating and neural GCs, negative feedback and resilience have not been tested under natural conditions.

The 2017 Atlantic hurricane season produced three highly destructive storms: Hurricanes Harvey, Irma and Maria. On 10 September 2017, Hurricane Irma made US landfall, first in the Florida Keys, then again in Marco Island-Naples, Florida on 10 September 2017. Hurricane Irma was the largest and strongest Atlantic hurricane ever recorded and was the longest-lasting Category 5 hurricane, when it devastated Northern Caribbean islands and South Florida resulting in 134 human deaths (Zolnikov 2018). The eye of Hurricane Irma passed ~112 km west of our study sites in Central Florida, where it interrupted our study on the behavior and stress physiology of the highly invasive lizard, the brown anole (*Anolis sagrei*) in both an urban site in Orlando, and a nearby forest site. Observations and

samples collected up to immediately prior to the hurricane provided baseline data to test the physiological and behavioral impacts of Hurricane Irma.

The brown anole, *A. sagrei* was introduced from its native range in Cuba and the Bahamas, into the USA through Florida where they have thrived and are now by far the most common lizard. Like other *Anolis*, the males of this species defend small territories (~3–4 m<sup>2</sup>) using visual displays involving both stereotyped physical movements like pushups and extensions of their fanned throat fan or dewlap (Schoener and Schoener 1982). As a diurnal species, *A. sagrei* relies on visual communication, which makes them susceptible to changes within the landscape that can interfere with their ability to signal at rivals and potential mates beyond their territories. Interestingly, counteracting this needs to be conspicuous, the abundant *A. sagrei* is also heavily preyed upon by birds and other visual predators (McLaughlin and Roughgarden 1989; Cantwell and Forrest 2013). This makes *A. sagrei* an excellent behavioral model to investigate how even modest changes in a landscape brought about by hurricane activity can alter the behavior of this species.

Pilot research has identified that the *A. sagrei* from the urban Orlando site consistently secreted less CORT during capture and handling (i.e. had lower stress reactivity) than those from the forested site (see Supplementary Figure). Multiple factors may explain these observations in CORT secretion include genetic variation derived from different native source populations (Kolbe, Larson, and Losos 2007; Kolbe et al. 2007, 2008) or site differences in ecological conditions not directly associated with aspects of urbanization, such as food availability or population density (Heiss, Clark, and McGowan 2009; Blondel et al. 2016). However, the most cited explanation is that urban lizards may attenuate their stress reactivity as a response to coping with daily stressors in the urban environment that end up being inconsequential, thereby saving their energetic investment for reproduction rather than survival (French et al. 2018). To reduce the CORT response, a robust negative feedback is necessary to return levels to baseline thus avoiding a prolonged and potentially detrimental exposure to GCs including possible neurobiological effects (Wingfield and Sapolsky 2003; Joëls 2018).

An attenuated CORT response during stress may indicate an increased resilience to an unpredictable environmental challenge, such as Hurricane Irma. Thus, we tested the hypothesis that the attenuated stress response in the urban *A. sagrei* population will translate into a more robust negative feedback and faster recovery to a post-hurricane Irma CORT concentration in both the blood and brain, compared to the forest anole population. Furthermore, we also tested whether any increases in CORT concentration will be associated with changes in behavior, such as an increase in avoidance (Atwell et al. 2012; Seltmann et al. 2012), or decreases in territoriality (Kabelik, Weiss, and Moore 2008; Fokidis, Orchinik, and Deviche 2011). The unique circumstances of this study have afforded us an opportunity to use our urban-forest comparison to test hypotheses regarding how land use impacts susceptibility to natural disasters and to discern some of the underlying animal physiology and behavior that can promote resilience in this context. Although, inclement, even severe, weather is generally assumed to induce only a transient effect on stress physiology (Romero, Reed, and Wingfield 2000), this has been very rarely directly studied. However, in the context of hurricanes this research can potentially inform biologists interested in both urban ecology and animal invasions.



**Figure 1:** Location of study sites impacted by Hurricane Irma. These areas suffered substantial damage including extensive tree damage and debris accumulation on the ground as well as flooding from excessive rainfall

## Materials and methods

### Study sites

We sampled *A. sagrei* from both urban and forested sites in Central Florida. The urban site was in downtown Orlando and centered around Lake Lucerne (Fig. 1). The urban site is bordered to the north by commercial properties, parking lots and a highway and to the south by commercial and some residential properties with the lake is bisected by a four-lane road (Orange avenue). The forest site was located in the Tosohatchee Wildlife Management Area ~40 km east of our urban site and bordering the St Johns River (Fig. 1), which contains a mixture of open and forested habitats typical of central Florida including hydric hammocks, cypress domes, upland sandhill and pine flatwood forests.

### Weather data

To assess the effects of Hurricane Irma in our region, we obtained weather data from the National Climate Data Center

online database (<https://www.ncdc.noaa.gov/cdo-web>). We used data precipitation (mm), air temperature ( $^{\circ}\text{C}$ ) and wind speed (km/h) data from the US1FLOR0023 weather station, located in Union Park, Florida, ~14 and 12 km from the urban and forest sites, respectively. We accessed weather data from these stations that both collected measurements daily from 1 September to 15 December 2017.

### Debris survey

Classified as a tree-ground ecomorph, *A. sagrei* is characterized by a tendency to perch on and defend the lower trunk of trees and rocks immediately surrounding tree trunks (Mattingly and Jayne 2004). Thus, we reasoned that the accumulation of fallen debris during the hurricane may influence territorial behavior in this species by either adding more perch sites for displays or disrupting territories that must be reestablished. To obtain a quantifiable estimate of this hurricane impact, we measured debris fall using a transect approach. In both sites, we established 10 separate non-overlapping 30-m transects in

randomized *a priori*, by throwing a dart on a map but accessible (i.e. not inundated) locations and every fallen tree or individual broken tree branch, including palm leaves, that crossed the transect line was counted. This method provided a rapidly measured, yet reliable estimate of the hurricane impact on the landscape. All transects were surveyed six times each at both the urban and the forest site from 2 September to 15 December 2017, including 2–3 days directly before and after the hurricane.

### Flight initiation distances

High CORT concentrations have been associated with increases in avoidance behaviors (Atwell et al. 2012; Seltsmann et al. 2012). Flight initiation distance (FID) is the minimal distance a human can approach an animal, before it takes flight and this metric has been used as an integrated measure of risk assessment, fear and overall tolerance to human presence (Blumstein 2003; Lin et al. 2012; Mikula 2014). Based on escape theory, FID will increase with overall predation risk and decrease as the cost of escape increases (Cooper et al. 2003). Urban animals often exhibit smaller FIDs compared to their rural conspecifics and this is largely attributed to habituation of animals to humans in urban areas (Valcarcel and Fernandez-Juricic 2009; Lin et al. 2012; Uchida et al. 2016). For both the forest and urban site, adult male anoles ( $n = 15$  on each sampling date) were first located, and then quietly approached at a steady and slow pace until they fled. Upon fleeing, the Euclidean distance between the researcher and the anole's final position was measured using a forestry tape measure. Anole FIDs were recorded at both sites on seven sampling dates from 2 September to 15 December 2017.

### Territorial behaviors

Male *Anolis* produce complex, but stereotypical social behaviors including head bobbing, pushups and most characteristically, extensions of their dewlap, a large flap of skin under the throat (McMann and Paterson 2012; Driessens et al. 2015). These social displays can escalate into territorial challenges between males and may precede fighting. At both sites, adult male anole lizards ( $n = 15$  on each sampling date) were located and focal observations were made using binoculars for 10 min each. During this time, the duration of time spent engaged in intraspecific interactions was recorded, as were the number of dewlap extensions during the interaction. Behavioral observations were recorded at both sites on seven sampling dates from 2 September to 15 December 2017.

### Animal capture and blood sampling

All animal handling and procedures were approved by the Rollins College Institutional Animal Care and Use Committee, protocol #2511a. Adult male anoles ( $n = 8$  for each site on seven sampling days between 2 September to 15 December 2017) were captured using fishing line snares between 1000 and 1300 h. Within 3 min of initial sighting and capture (initial blood sample), 15–30  $\mu$ l of blood was collected by rupturing the orbital sinus with a heparinized capillary tube with blood collection taking no longer than 30 s. From the initial blood sample, thin blood smears were made in the field on glass microscope slides using a drop ( $\sim 5 \mu$ l) of blood. Anoles were then held in a clean opaque cloth bag for 20 min after which another blood sample ( $\sim 10$ – $20 \mu$ l) was collected from the other eye to provide the stress-induced sample. Collectively, these samples permit us to assess the change in circulating CORT concentrations during

capture/handling stress. All blood was stored on ice until transported back to the laboratory. Anoles were then weighed using a Pesola scale ( $\pm 0.5$  g), and measurements of the snout-vent length (SVL), head depth (HD) and head length (HL) were taken, and the presence of any scars or injuries, including caudal autotomy (i.e. tail breaks), was noted. At the laboratory, plasma was separated using centrifugation (10 min at 2500 rpm) and stored at  $-20^\circ\text{C}$  until assayed.

### Dexamethasone suppression tests

To assess the efficacy of the negative feedback, an additional sample of male anoles ( $n = 12$ ) from each site was sampled on two occasions: 11 August and 29 September (forest) and 15 August and 27 September (urban). Lizards were bled within 3 min of capture as above, and then quickly received an intraperitoneal injection of 50  $\mu$ l of DEX (at a 1.6  $\mu$ g/g dose in 0.75% NaCl or saline;  $n = 6$ ), which is a synthetic agonist of the GC receptor widely used to test resistance of the stress axis to negative feedback (Fokidis and Deviche 2011). As a control, another sample of anoles ( $n = 6$ ) received a 50- $\mu$ l injection of the 0.75% NaCl saline vehicle. Lizards were then held in a cloth bag, and after 20 min a second blood sample was collected. The treatment effect was assessed by comparing the percent change in plasma CORT concentrations between pre- and post-treatment with DEX.

### Brain collection and microdissections

To assess neural CORT levels, a subsample of male anole lizards ( $n = 8$  each site sampled on 7–8 September, 12–13 September and 17, 19 October) were captured and sacrificed within 3 min using rapid decapitation, to avoid any acute steroid changes with capture. Trunk blood was collected in a heparinized capillary tube and stored on ice, and the brain dissected out within 2 min and frozen in the field with dry ice. Brain samples were transferred to the lab and stored at  $-80^\circ\text{C}$  until processing.

Anole brains were microdissected to measure neural CORT concentration within specific stress-sensitive brain regions isolated using a modified Palkovits punch technique (Palkovits 1983; Charlier et al. 2010). These micropunches can sample a finer resolution of the brain, such as individual nuclei of the hypothalamus, however due to the small size of the *Anolis* brain we opted for sampling broader brain regions to maximize CORT detection and quantification.

Brains were first sectioned on a cryostat through the coronal plane at 200  $\mu$ m with positioning determined using the *Anolis carolinensis* atlas (Greenberg 1982). All sections for each individual were kept frozen and then mounted onto a slightly warmed microscope slide, which helped to affix the tissue. Slides were transported onto ice and under a dissecting microscope were punched through specific brain regions using a specialized punch apparatus (Stoelting #57401) with a nominal diameter of 50  $\mu$ m. The brain regions targeted were the hypothalamus (HYP); the amygdala (AMY); and the dorsal and medial cortex (CX), a homolog to the mammalian hippocampus (Rosen et al. 2002; Singletary, Hayworth, and Delville 2010). These regions were selected for their known functional roles in mediating stress, anxiety and social behaviors in anoles (Rosen et al. 2002; Singletary, Hayworth, and Delville 2010). Additional brain regions were also sampled including the dorsoventricular ridge (DVR), the lateral forebrain bundle (LFB) and the cerebellum (CB), and these served as 'control' regions where localized CORT increases are not expected due to a lack of functional roles for these regions during stress. Punches were made through both

hemispheres and expelled into a frozen microcentrifuge tube. For all brain regions, multiple punches could be made over successive sections, thus all punches from the same region per individual were pooled together to maximize CORT detection. The number of punches that were pooled for each individual for analysis of each region was HYP ( $n=8$ ), AMY ( $n=6$ ), CX ( $n=9$ ), DVR ( $n=12$ ), LFB ( $n=6$ ) and CB ( $n=10$ ). Micropunches were stored at  $-80^{\circ}\text{C}$  until steroids were extracted.

### CORT extraction and analysis

To extract CORT from brain micropunches, we used solid-phase extraction with C18 columns as previously described (Fokidis, Prior, and Soma 2013). This method yields a high and consistent steroid recovery and effectively removes potentially interfering lipids (Charlier et al. 2010). Briefly, brain punches were homogenized in a bead homogenizer (settings: 12 m/s for 30 s, Omni Bead Ruptor 24, Omni International, Kennesaw, GA, USA) in 1 ml of ice-cold 80% HPLC-grade methanol (MeOH) and left overnight at  $4^{\circ}\text{C}$ . Samples were centrifuged (3000 g) the following day and the supernatant was collected to which was added 10 ml of  $\text{dH}_2\text{O}$ , prior to loading on C-18 columns. Here,  $10\ \mu\text{l}$  of the remaining tissue pellet (duplicates of  $5\ \mu\text{l}$ ) was used to correct the neural steroid concentrations obtained from micropunches by analyzing sample protein concentrations with Coomassie Plus Protein Assay reagent (Pierce, Rockford, IL, USA) according to previous studies (Charlier et al. 2011; Fokidis, Prior, and Soma 2013). Protein content was  $5.35 \pm 0.87\%$  of the estimated wet weight of micropunches, which is comparable to previous reports in other species (Pradhan et al. 2010; Fokidis, Prior, and Soma 2013). To detect potential contamination, all solid-phase extraction runs included a water blank run alongside samples. Columns were primed with 3 ml of 100% MeOH and then equilibrated with 10 ml  $\text{dH}_2\text{O}$ . Samples were then loaded on the column. This was followed by an interference elution with 10 ml 40% MeOH; this elution aimed to remove interfering lipids and glucuronidated and sulfated steroids. Finally, steroids were eluted with 5 ml 90% MeOH. Samples were dried at  $40^{\circ}\text{C}$  in a vacuum centrifuge (Thermo Electron SPD111V Speedvac; Thermo Scientific) and stored at  $4^{\circ}\text{C}$  until assayed.

Plasma was separated from the whole blood via centrifugation (10 min at 2500 rpm) and stored at  $-20^{\circ}\text{C}$  until assayed. Dried extracts from brain micropunches were resuspended with  $60\ \mu\text{l}$  of absolute ethanol (3% of total resuspension volume) in the assay buffer provided with the commercial enzyme-linked immunoassay for CORT (Arbor Assays Inc., Ann Arbor, MI, USA). This CORT assay was validated for anoles by (1) demonstrating parallelism between serially diluted samples and the standard curve, (2) showing a high recovery of exogenous CORT (91–93% recovery) and (3) a low recovery of samples stripped of CORT with dextran-coated charcoal (1–5% recovery). Steroid recovery was previously determined by spiking samples with known amounts of CORT and comparing them to un-spiked samples ( $n=3$  for each of plasma and brain tissue). Recovery of CORT was  $94 \pm 4.23\%$  in plasma and  $91 \pm 3.14\%$  in brain tissue. Samples were corrected for recovery where applicable. All plasma samples were assayed in duplicate, but the small-sized brain punches were run as singletons to maximize detection of CORT. Plasma samples were run separately from brain samples, but initial and stress-induced blood samples from the same individuals were run on the same 96-well plate ( $N=9$  plates, 368 samples total). CORT concentrations were then calculated by interpolation from the standard curves present on each plate

using GraphPad Prism version 4 (La Jolla, CA, USA). The sensitivity of the CORT assay ranged from 3.1 to 5.5 pg/ml and the mean intra-assay and inter-assay coefficients of variation were 6.1 and 10.8%, respectively.

### Blood smears and the heterophils to lymphocytes ratio

Blood smear slides were air-dried at ambient temperature and stored under vacuum until fixation. Blood smears were then fixed for 10 min in absolute methanol and stained using the Giemsa method (Fokidis, Greiner, and Deviche 2008; French, Fokidis, and Moore 2008). Stained smears were dehydrated for  $\sim 1$  week under partial vacuum and then cleared using xylene. Slides were coverslipped and sealed using Cytoseal 60 (VWR, San Francisco, CA, USA) for long-term storage. The ratio of heterophils to lymphocytes (H/L) has been used as crude indicators of the innate and adaptive immune defenses, respectively (Vleck et al. 2000; Krams et al. 2012). Increases in the H/L ratio often occur concurrently with elevated GC levels, which has suggested that stress suppresses adaptive immunity (i.e. lymphocytes) in favor of innate immunity (i.e. heterophils). Thus, H/L ratio has been used as a supplemental measure of chronic stress exposure (Vleck et al. 2000), although this idea has been challenged. The H/L ratio was determined as previously described (Fokidis, Greiner, and Deviche 2008) by examining randomly selected and non-overlapping microscope fields for each slide at  $400\times$  magnification and counting heterophils and lymphocytes until a total of 50 cells is reached. This was repeated three times for each blood smear, and the mean H/L ratio was calculated as used in subsequent analysis.

### Statistical analyses

All data were tested for normality of distribution using Kolmogorov-Smirnov test and for homoscedasticity using Levene's test of equal variance. When necessary, data were either arcsine square root (H/L ratio and the percentage response to DEX) or log-transformed (FID data) prior to analysis to satisfy the above assumptions.

A body condition index was developed from the residuals of an ordinary least squares regression of each morphometric measure (SVL, HL and HD) on body mass that were standardized to zero. The regression of SVL on mass had the highest goodness of fit ( $R^2 = 0.88$ ;  $n = 112$ ;  $P = 0.023$ ) compared to HL-mass ( $R^2 = 0.71$ ;  $n = 112$ ;  $P = 0.038$ ) and HD-mass ( $R^2 = 0.68$ ;  $n = 112$ ;  $P = 0.041$ ). Residuals of SVL-mass provided the most conservative metric for assessing mass of potential energy stores that was not explained by body size and has been used successfully in *A. sagrei* (Cox and Calsbeek 2015). Thus SVL-mass residuals were used as the body condition index for subsequent analysis. As SVL in anoles is associated with numerous traits (Campbell and Echternacht 2003), we also included SVL as a separate covariate in subsequent analyses.

The goal was to analyze the effects of Hurricane Irma on anole behavior (FIDs, the number and duration of interactions between individuals and the number of dewlap extensions) and physiological traits (H/L ratio, plasma and brain CORT). As all data were collected from two sites over several weeks, but without sampling the same individuals through time, we used a general linear model analysis of variance (GLM ANOVA) for behavior, blood and brain data. To test for population differences in the response to DEX treatment, we instead used repeated measures ANOVA (RM ANOVA), which incorporated pre- and post-injection CORT concentrations as the within-subject

factors (dependent variable). For all ANOVA models mentioned above, both site and sampling dates were included as fixed effects, and the time of day, body condition and SVL, as well as the relevant interaction of site  $\times$  date were all included as covariates in these models. To investigate neural CORT concentration, we also include brain region as a main factor, as well as the interaction between brain region  $\times$  site and brain region  $\times$  date and brain region  $\times$  site  $\times$  date. Fisher's least significant differences (LSD) tests were used for *post hoc* analysis to compare between sites on specific dates, or between dates of specific interest, such as immediately before and after the hurricane. Differences in debris fall between sites were similarly compared using GLM ANOVA with site and date as main effects.

To assess the relationships between specific behavioral and physiological traits, we used Pearson's correlations. All data are presented as means  $\pm$  standard error of the mean, and analyses were conducted using SPSS for Windows: version 22 (IBM Corp., Armonk, NY, USA).

## Results

### Local impacts of Hurricane Irma

Hurricane Irma completely engulfed the state of Florida (Fig. 2A) and our study sites were located in front right quadrant of the hurricane. At the urban site, Hurricane Irma generated significant debris from downed trees and branches and the rainfall raised Lake Lucerne's water levels resulting in complete inundation of Orange avenue and a flooded shoreline  $\sim$ 10m from the original location (Fig. 2B). These flooded conditions persisted for  $\sim$ 5 days following the hurricane, before water levels began to recede.

At the forest site, Hurricane Irma expectedly generated more debris than the urban site, as well as flooding from both substantial rainfall and the overflow of the St Johns River (Fig. 2C). Areas near the St John's river were dangerous and inaccessible, but areas sampled prior to the hurricane were largely accessible, though flooded. Most of the forest site remained flooded up to 4 weeks post-hurricane, but the flood zone began to recede in late October 2017.

Air temperatures expectedly declined over the study period, with only a brief 1-day decline with the hurricane passing (Fig. 2D). As expected, Hurricane Irma generated high precipitation, in addition to the daily regular rainfall, but as the study period progressed conditions became drier (Fig. 2D).

Wind speeds during Hurricane Irma were almost double that observed at any other time during the study period, and as expected generated significant debris from uprooted trees and downed branches at both sites (Fig. 2E). The urban site had expectedly less debris than the forest site (site:  $F = 17.75$ ,  $P \leq 0.001$ ; Fig. 2E) but at both sites, debris fall decreased during the study period (date:  $F = 5.80$ ,  $P = 0.024$ ; Fig. 2E). The faster decrease at the urban site is attributed to debris clearance by city officials within a month of the hurricane, although differences in the rate of debris removal were not statistically significant (site  $\times$  date:  $F = 9.84$ ,  $P = 0.004$ ).

### Hurricane Irma changed *Anolis* behavior

The *A. sagrei* were more approachable (i.e. had lower FIDs) in the urban compared to the forest site (site:  $F = 4.69$ ,  $P = 0.008$ ; Fig. 3A). Although date was not significant (date:  $F = 1.96$ ,  $P = 0.077$ ), for  $\sim$ 2 weeks following the hurricane, forest anoles had higher FIDs than urban conspecifics, the latter not effected

by the hurricane (Fig. 3A). Time of sampling also had no effect on FID (time:  $F = 0.04$ ,  $P = 0.999$ ). Interestingly of note, following the hurricane at both sites, many more anoles were observed possibly due to some lizards being displaced from flooded territories, resulting in a higher density of animals overall. Thus, intraspecific interactions between anoles were common and easily observed following Hurricane Irma.

The time spent engaged in intraspecific confrontations differed between study sites (site:  $F = 3.66$ ,  $P = 0.019$ ) and across the study period (date:  $F = 2.40$ ,  $P = 0.027$ ; Fig. 3B). There was also a significant effect of the interaction (site  $\times$  date:  $F = 7.34$ ,  $P = 0.003$ ; Fig. 3B). Immediately prior to the hurricane, urban anoles spent more time in confrontation than forest anoles (LSD  $P = 0.032$ ), but this pattern was reversed immediately following the hurricane (LSD  $P = 0.016$ ; Fig. 3B). Within 2 weeks, no differences between sites were detected and within a month after the hurricane urban anoles spent more time confrontations than forest lizards (Fig. 3B).

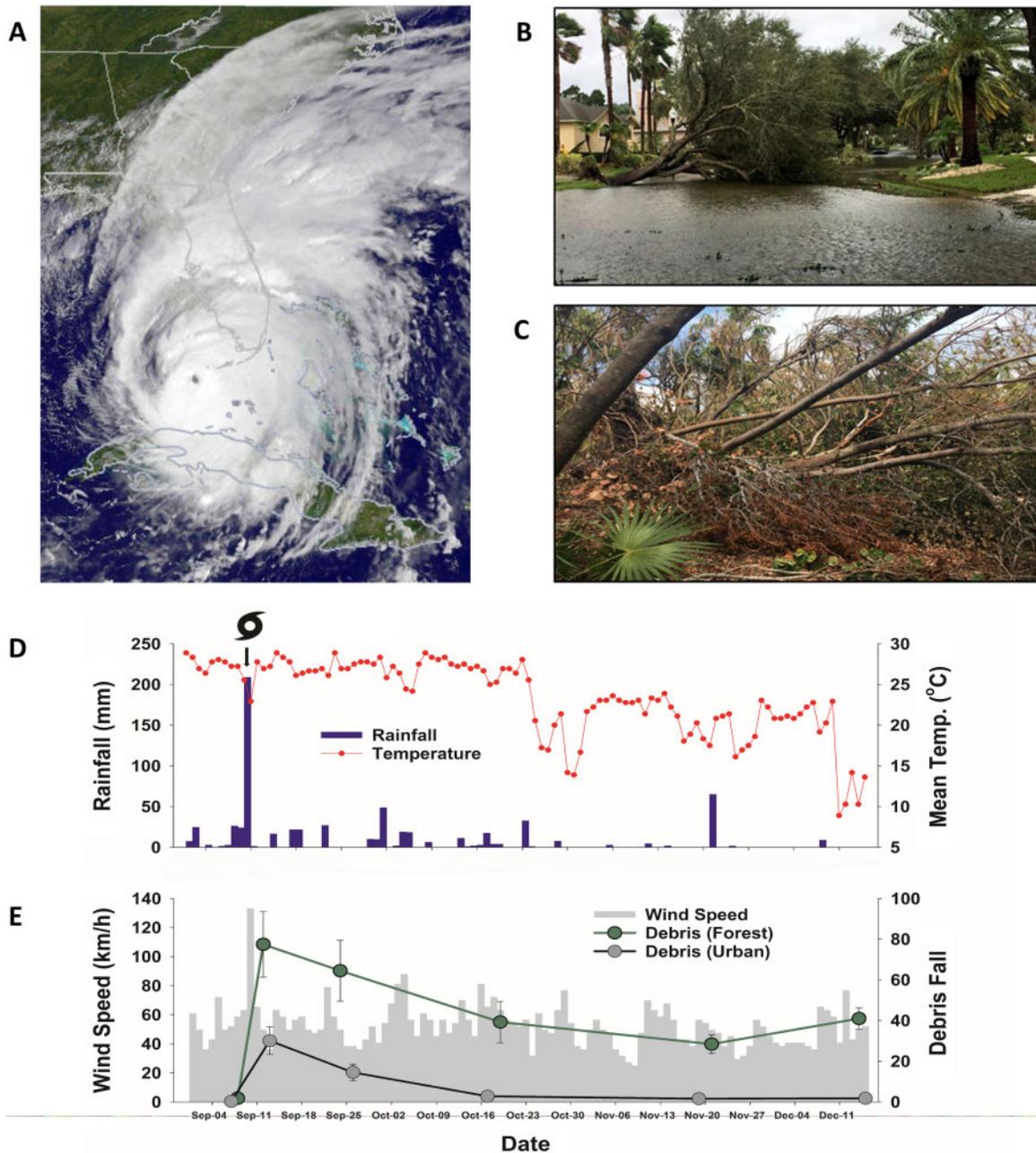
Dewlap displays were more common in forest than urban anoles (site:  $F = 3.91$ ,  $P = 0.021$ ) and were generally more common early in the study period than later (date:  $F = 3.46$ ,  $P = 0.016$ ; Fig. 3C), although this pattern was largely seen in forest anoles (site  $\times$  date:  $F = 16.11$ ,  $P \leq 0.001$ ). Interestingly, dewlap displays were more prevalent following the hurricane in forest anoles for up to 1 month compared to urban anoles (12 September to 17 October; all LSD  $P \leq 0.034$ ).

### Hurricane effects on *Anolis* stress physiology

Body condition did not significantly vary across the sampling period (date:  $F = 0.38$ ,  $P = 0.615$ ) or between sites (site:  $F = 0.27$ ,  $P = 0.734$ ). Similarly, SVL also did not differ (site:  $F = 0.91$ ,  $P = 0.511$ ; date:  $F = 1.04$ ,  $P = 0.231$ ).

Preliminary research (see Supplementary Figure) revealed that 20 min corresponded to the maximal plasma CORT levels observed in anoles during capture and handling stress. Here, baseline plasma CORT concentrations were similar between sites (site:  $F = 0.85$ ,  $P = 0.384$ ; Fig. 4A) and across the sampling period (date:  $F = 1.73$ ,  $P = 0.175$ ; Fig. 4A). Urban anoles exhibited an attenuated CORT stress response compared to forest lizards (site:  $F = 3.16$ ,  $P = 0.037$ ; Fig. 4A). Stress-induced CORT concentrations varied throughout the study period (date:  $F = 5.38$ ,  $P = 0.014$ ) and this differed between sites (site  $\times$  date:  $F = 5.13$ ,  $P = 0.026$ ; Fig. 4A). *Post hoc* analysis revealed that urban lizards had lower stress-induced CORT levels than forest lizards before the hurricane (LSD all  $P \leq 0.046$ ), but no differences between sites were observed the 2 days following the hurricane (12–13 September: LSD  $P = 0.74$ ). Interestingly, these site differences resurfaced on the following sample date (24–25 September: LSD  $P = 0.015$ ) Fig. 4A). Body condition was not correlated to baseline CORT concentrations ( $r = 0.12$ ,  $P = 0.284$ ); however, a significant overall negative relationship between stress-induced CORT concentrations and body condition was observed ( $r = -0.21$ ,  $P = 0.036$ ).

In testing negative feedback, administration of DEX significantly suppressed CORT concentrations compared to saline-treated controls (RM ANOVA treatment:  $F = 2.037$ ,  $P = 0.028$ ; Fig. 4B). This DEX effect did vary between sampling dates (date:  $F = 1.63$ ,  $P = 0.049$ ) and across sites (site:  $F = 2.38$ ,  $P = 0.006$ ), and this was entirely due to forest anoles having increased CORT levels after DEX treatment 2 weeks' post-hurricane (site  $\times$  date:  $F = 3.70$ ,  $P = 0.028$ ; LSD  $P = 0.033$ ), whereas CORT levels were suppressed by DEX treatment at other sampling dates at both sites (all LSD  $P > 0.155$ ). The response to DEX treatment did not differ



**Figure 2:** Local weather conditions and environmental impacts of Hurricane Irma throughout the sampling period (September–December 2017). (A) Satellite image of Hurricane Irma engulfing Florida taken on 10 September 2017. Photo was obtained from [www.nasa.gov](http://www.nasa.gov). (B) Photos of freshwater flooding in a residential area and (C) fallen debris accumulated in Orlando. Weather data collected from stations located between an urban (Lake Lucerne, Orlando) and a forest site (Tosohatchee Wildlife Management Area) including (D) daily mean air temperatures and total daily rainfall and (E) mean wind speeds and the amount of debris fall counted along 10 transects (30 m in length). Timing of Hurricane Irma is indicated by arrow and hurricane symbol. Values for urban (gray points) and forest (green points) anoles indicate means and error bars indicate SEM

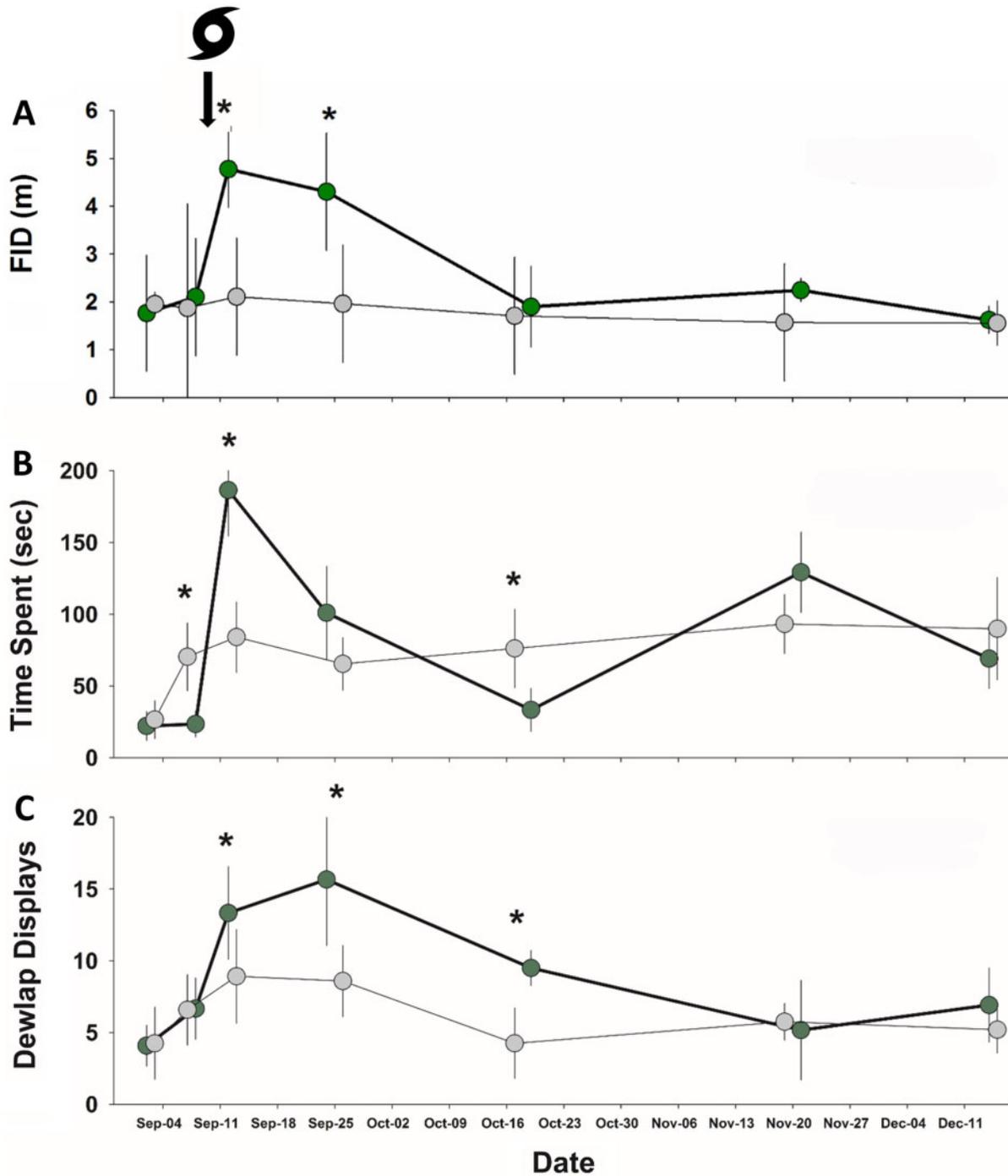
with time of day, SVL or body condition (all RM ANOVA  $P \geq 0.093$ ).

A significant overall negative relationship between stress-induced CORT concentrations and body condition was observed ( $r = -0.312$ ,  $P = 0.036$ ; Fig. 4C). The H/L ratio significantly decreased over the course of the sampling period (date:  $F = 3.23$ ,  $P = 0.040$ ; Fig. 5); however, there were no differences between urban and forest anoles (site:  $F = 0.143$ ,  $P = 0.811$ ). There were no correlations between plasma CORT and H/L ratio (baseline:  $r = 0.101$ ,  $P = 0.773$ ; stress-induced:  $r = 0.063$ ,  $P = 0.813$ ), but

interestingly, a slight positive correlation between SVL and H/L ratio was observed ( $r = 0.215$ ,  $P = 0.047$ ).

### Hurricane and the brain

There was a significant effect of brain region on CORT concentrations (GLM ANOVA, brain region:  $F = 6.45$ ,  $P \leq 0.001$ ). Thus, CORT concentrations varied across the different regions of the brain that were sampled (Fig. 6A). As expected, neural CORT levels were at a concentration that was an order of magnitude

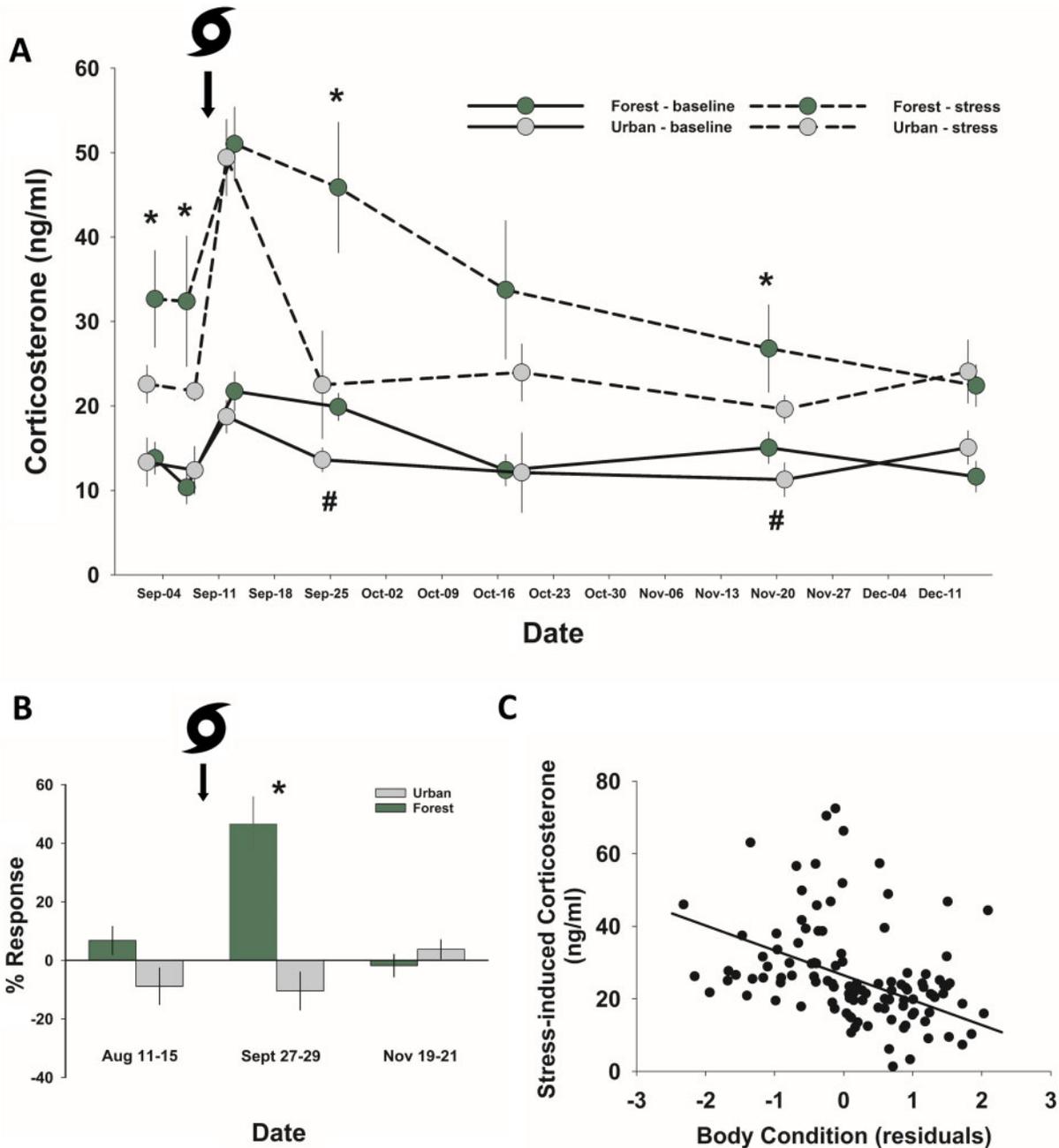


**Figure 3:** Behavioral changes in urban and forest brown anoles (*Anolis sagrei*) in response to Hurricane Irma. (A) Avoidance behavior of anoles as measured by FID, the distance an animal flees from an approaching threat, in this case the researcher. Territorial behaviors observed in focal male anoles sampled before and after Hurricane Irma, including (B) the time spent interacting with conspecifics and (C) the number of dewlaps displayed during a 10-min observation period. Timing of Hurricane Irma is indicated by arrow and hurricane symbol. Values for urban (gray points) and forest (green points) anoles indicate means and error bars indicate SEM. Statistical differences between sites are indicated by \* $P < 0.05$

lower than present in circulation. There were also significant main effects of both site (site:  $F = 1.86, P = 0.050$ ) and sampling date (date:  $F = 2.09, P = 0.043$ ). The effects on the various brain regions differed across sites (brain region  $\times$  site:  $F = 6.86, P = 0.016$ ), but not across the sampling period (brain region  $\times$  date:  $F = 0.49, P = 0.32$ ). There was a significant effect of region

across both sites and sampling date (brain region  $\times$  site  $\times$  date:  $F = 2.06, P = 0.041$ ).

Post hoc analyses reveal that prior to Hurricane Irma all brain regions showed similar amounts of neural CORT with no differences between sites (Fig. 6B–C; all LSD  $P \geq 0.206$ ). Immediately following the hurricane, several regions showed CORT increases



**Figure 4:** Decreased plasma CORT responses to stress in urban compared to forest brown anoles (*Anolis sagrei*) following Hurricane Irma. (A) Baseline (solid lines) and acute (20 min) stress concentrations (dashed lines) of corticosterone in both urban and forest anole lizards sampled before and after Hurricane Irma. (B) Percentage of plasma corticosterone response to dexamethasone treatment in both urban and forest anole lizards sampled before and after Hurricane Irma. Comparisons are between plasma samples collected at baseline and those sampled 20 min following injection with dexamethasone, a GC agonist that simulates negative feedback. (C) Negative correlation between stress-induced CORT concentrations and anole body condition obtained from standardized residuals of snout-vent length on body mass ( $r = -0.12$ ,  $P = 0.036$ ). Timing of Hurricane Irma is indicated by arrow and hurricane symbol. Values for urban (gray points) and forest (green points) anoles indicate means and error bars indicate SEM. Statistical differences in plasma corticosterone between sites are indicated by \* for baseline and # for stress at  $P < 0.05$

above pre-hurricane levels including the AMY (Figs. 6B; LSD  $P = 0.037$ ), CX (Fig. 6D; LSD  $P < 0.001$ ) and LFB (Fig. 6E; LSD  $P = 0.042$ ), whereas the DVR (Fig. 6C; LSD  $P = 0.611$ ) and HYP (Fig. 6F; LSD  $P = 0.273$ ) did not show an increase. Following the hurricane, forest anoles had higher CORT concentrations compared to urban conspecifics in three of these brain regions: AMY (Fig. 6B;  $P = 0.028$ ), CX (Fig. 6D;  $P = 0.019$ ) and LFB (Fig. 6E;  $P = 0.025$ ). Interestingly, the site difference was retained in the

AMY a month later (Fig. 6B; LSD  $P = 0.004$ ), was lost in the CX (Fig. 6D; LSD  $P = 0.285$ ) and was reversed in the LFB (Fig. 6E; LSD  $P = 0.047$ ).

Blood collected from these anoles prior to brain collection did not differ between sites or dates (all  $P > 0.32$ ), and there was no significant correlation between plasma CORT concentrations and neural CORT for any brain region on any date (range of Pearson's  $r = -0.073$ – $0.101$ ; all  $P \geq 0.548$ ). There were also no

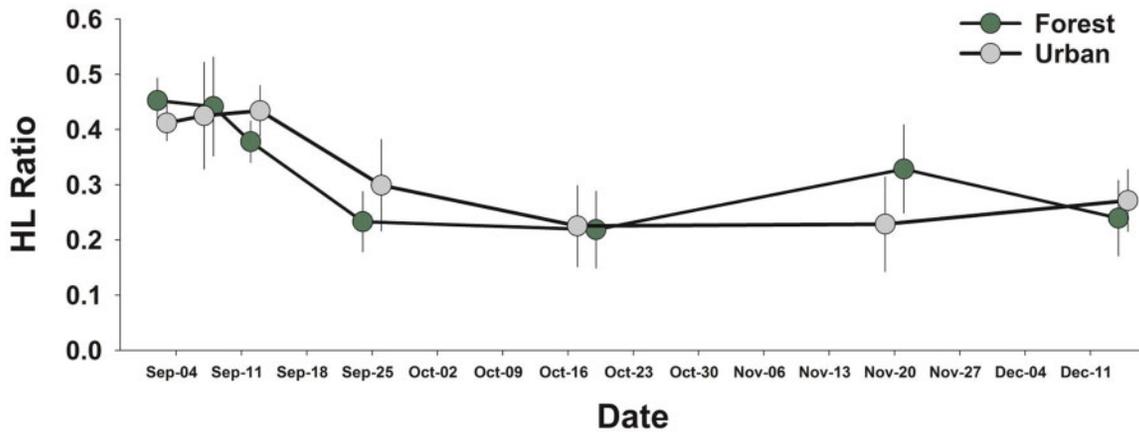


Figure 5: Variation in the ratio of heterophils to lymphocytes (HL ratio) in both urban and forest brown anoles (*Anolis sagrei*) sampled before and after Hurricane Irma. Timing of Hurricane Irma is indicated by arrow and hurricane symbol. Values for urban (gray points) and forest (green points) anoles indicate means and error bars indicate SEM

relationships between neural CORT and body condition, SVL or H/L ratio (all  $P \geq 0.734$ ).

## Discussion

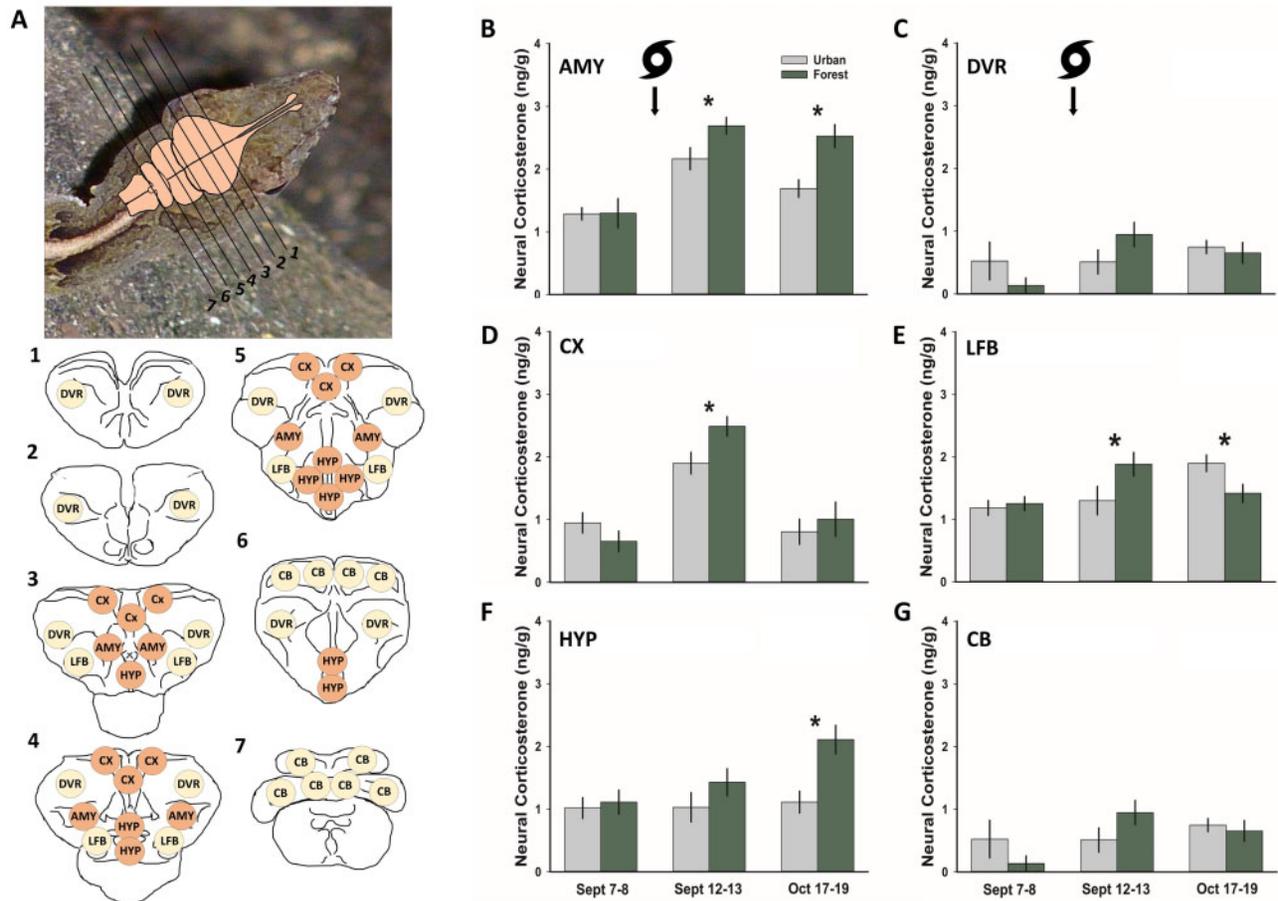
Hurricanes and other natural disasters have profound impacts on biodiversity (Meshaka 1993; Schoener, Spiller, and Losos 2004; Schoener and Spiller 2006), ecosystem processes (Ostertag, Scatena, and Silver 2003; Spiller and Schoener 2007; Feehan, Scheibling, and Lauzon-Guay 2012) and even evolution (Donihue et al. 2018, 2020). Yet studies have sampled the weeks and months following a natural disaster, thus missing the ephemeral and immediate effects. We investigated Hurricane Irma's impacts on territoriality, avoidance behavior and the CORT secretion in both an urban and forest population of *A. sagrei*. Within days of the hurricane, forest anoles exhibited a higher degree of avoidance (i.e. FID) and increased their antagonistic behaviors, such as dewlap displays, compared to urban conspecifics. Concurrently, urban anoles had an attenuated CORT response to capture stress compared to forest lizards, which was explained by a robust negative feedback in urban lizards. Furthermore, CORT levels were elevated in particular brain regions after the hurricane differently between sites. Although the physiological and behavioral changes observed were short-lived, the rate of recovery to a pre-hurricane baseline was faster in the urban anoles. Urban animals often exhibit attenuated responses to stress (French, Fokidis, and Moore 2008; Fokidis, Orchinik, and Deviche 2009; Atwell et al. 2012), however, a recent meta-analysis of the HormoneBase database (Vitousek et al. 2018) suggested that CORT concentrations alone cannot diagnose the effects of urbanization on birds or reptiles, and instead research should be supplemented with other behavioral and physiological metrics (Injaian et al. 2020). The results in this study are consistent with an urban resilience hypothesis where HPA attenuation resulting from an urban existence may permit faster recovery to drastic environmental changes, from hurricanes.

The logistics of working shortly after a major hurricane prevented sampling more populations across the urban-forest continuum. Thus, the inferences drawn from this study are limited by our two-sample comparison. First documented in the 1880s, *A. sagrei* became established in Florida by the 1940s and likely reached our sites in the late 1960s to early 1970s (Kolbe et al.

2004), and genetic studies suggest that up to eight separate invasions have occurred (Kolbe et al. 2004), and thus our study populations may be derived from different stock populations. However, these lizards are found at high densities in a contiguous distribution between our study sites, thus interbreeding has likely diluted any genetic variation arising from these original source populations. Spatial replication in this study is difficult as it would require a highly coordinated effort to sample multiple locations soon after a hurricane, but the results of this study could be verified with temporal replication by using opportunities afforded by future hurricanes to test the urban resilience hypothesis.

Successful urban animals are noted for their tolerance to human presence compared to rural counterparts (Atwell et al. 2012; Mikula 2014). Abundant at both sites, *A. sagrei* was nonetheless more easily approachable at the urban site, than the forest as frequent, non-consequential human encounters have likely allowed urban lizards to habituate to humans quickly. Forest lizards had a non-significant trend suggesting a decreased tolerance after the hurricane. One explanation may be higher predation risk following the hurricane in rural anoles (Cooper et al. 2003; Diego-Rasilla 2003; Berger et al. 2007; Cooper 2010; Atwell et al. 2012), but this is difficult to quantify and test. Territorial interactions generally increased in forest, but not urban anoles, yet both experienced a short-lived increase post-hurricane. Territorial instability due to an influx of lizards whose own territories may have been flooded is one explanation, although this remains unknown. Interestingly, site differences persisted for a month, despite densities seemingly returning to pre-hurricane levels, thus lizard density is unlikely the sole driver of behavioral persistence in the forest anoles. Urban birds can be more territorial than rural ones as urban settings often select for behavioral boldness (Evans, Boudreau, and Hyman 2010; Scales, Hyman, and Hughes 2011), yet frequent interactions with the same neighbors may induce a habituation known as the 'dear enemy' effect (Paterson 2002; McMann and Paterson 2012). As flooding persisted longer in the forest site, unfamiliar individuals may have been present longer. Although, territorial displays are unrelated to prey availability in *A. carolinensis* (Stehle et al. 2017), variation in food availability is another potential factor to be considered.

Steroids are well-established regulators of *Anolis* social behavior (Tokarz et al. 1998; Husak et al. 2007; Dunham and



**Figure 6:** Neural corticosterone accumulation in urban and forest brown anoles (*Anolis sagrei*) before and after Hurricane Irma. (A) Relative coronal sections and specific locations of micropunches collected from specific brain regions either directly associated with stress (in ochre) or unassociated with stress (in beige). Numbers in photo correspond to the numbered sections and all brain regions of the same type were pooled together to insure corticosterone measurement. Brain regions sampled include (B) the amygdala (AMY), (C) the dorsoventricular ridge (DVR), (D) the dorsal and medial cortex (CX), (E) lateral forebrain bundle (LFB), (F) the hypothalamus (HYP) and (G) the cerebellum (CB). Timing of Hurricane Irma is indicated by arrow and hurricane symbol. Values for urban (gray bars) and forest (green bars) anoles indicate means and error bars indicate SEM. Statistical differences in plasma corticosterone between sites are indicated by \* $P < 0.05$

Wilczynski 2014). Exogenous CORT suppresses aggression in male *A. sagrei* (Tokarz 1987), and baseline levels are higher in the fall and winter nonbreeding periods and lower in the summer breeding season when more territorial (Husak et al. 2007; Dunham and Wilczynski 2014). No seasonality in CORT was observed, despite decreased behavioral activity in the winter. The attenuated CORT response of urban anoles is consistent with some urbanization studies (Bonier 2012). Hurricane Irma heightened CORT responses in both populations, but urban anoles recovered to their pre-hurricane levels faster than forest anoles, but as baseline concentrations did not differ interpretation is problematic. First, high baseline CORT should suppress territoriality resulting in either lower aggression in forest lizards or no behavioral differences between sites. These data suggest no direct relationship between plasma CORT and territorial behavior. Second, inclement weather has been traditionally viewed as an ‘acute stressor’ that does not induce long-term HPA effects (Romero, Reed, and Wingfield 2000). Only a single study has explored hurricane effects on the HPA axis. Within a month of Hurricane Rita, American alligators (*Alligator mississippiensis*) experienced very high plasma CORT concentrations due to severe osmotic stress from both a coastal saltwater intrusion and an ongoing drought (Lance et al. 2010). Here, the persistence of CORT in forest anoles also suggests ‘chronic stress’. In Central

Florida however, Hurricane Irma would likely be interpreted by an anole as just another rain and wind event followed by a 1–2-day drop in air temperatures. Prolonged flooding and debris better explain the longer lasting impact on forest anoles, since the floods receded much earlier and city officials cleaned the debris in urban site, unlike in the forest. Thus, CORT levels may be a response to the more persistent habitat effects of the hurricane in the forest compared to the city. Other studies linking weather to HPA function and fitness have been tied to specific environmental aspects, and not the weather event per se (Rogers et al. 1993; Romero, Reed, and Wingfield 2000; Bize et al. 2010; Romero and Wikelski 2010; Dupoué et al. 2018). A future test of the urban resilience hypothesis could replicate hurricane impacts and document how soon after clean-up the CORT levels recovered to baseline.

Administering DEX successfully suppressed the CORT response in all samples except for forest anoles right after the hurricane, which is consistent with our data on plasma CORT concentrations. Thus, forest anoles exhibited a desensitized HPA axis that may result from a compromised negative feedback. This is an understudied area in reptiles (but see Romero and Wikelski 2010), but studies using DEX across urban landscapes have been previously reported in birds (Fokidis and Deviche 2011) and even in human adolescents that survived

Hurricane Katrina compared to non-affected inhabitants (Pfefferbaum, Tucker, and Nitiéma 2015). Adjustments to living in the novel urban ecosystem are often associated with a declined acute stress response (Fokidis, Orchinik, and Deviche 2011; Atwell et al. 2012), which requires a robust negative feedback to return CORT levels to baseline once a stressor has passed. Thus, urban *Anolis* may be predisposed to handle sudden, but ephemeral hurricane impacts on their habitats resulting in an urban resilience.

Dysregulation of the HPA feedback elevates GC levels leading to deleterious effects on fitness (Bonier et al. 2009; Crespi et al. 2013). Here, we could not directly investigate fitness (i.e. survival or reproduction), but instead tested relationships between CORT and condition indices that can relate to fitness. Although body condition or SVL did not differ, lizards in better body condition generally had lower stress CORT concentrations. Similarly, no site differences in the H/L ratio was observed, despite chronic stress often suppressing lymphocyte production. The seasonal decline in H/L ratio later may relate to metabolic changes or decreased disease risk with colder temperatures. Together our data do not support Hurricane Irma imparting long-lasting maladaptive effects in either population.

Chronic CORT exposure can impact stress-sensitive brain nuclei (Shepard, Barron, and Myers 2000; Oitzl et al. 2010), although little research on brain CORT levels in free-living animals has been done. Here, we note a heterogeneous CORT distribution across the brain, but at concentrations an order of magnitude below circulation. There was little correlation between neural CORT that in circulation suggesting neural CORT accumulates over time in a region-specific manner. Thus, it is not simply reflective of CORT that recently entered the brain at time of sampling. Furthermore, only some brain regions showed CORT changes with the Hurricane and those regions closest to the blood-brain barrier did not change (i.e. CB), unlike deeper brain regions (i.e. CX or AMY). Importantly, the neural CORT increase was more pronounced in forest anoles following the hurricane in the AMY and CX, which are CORT sensitive regions in the mammalian and avian brain (Shepard, Barron, and Myers 2000; Gourley, Swanson, and Koleske 2013). In the AMY, excessive CORT increases fear-based behaviors in lab rodent models (Monsey et al. 2014) and this is analogous to observations of post-traumatic stress disorder in humans (Kaouane et al. 2012). The medial and dorsal CX area is the putative reptilian homolog of the hippocampus (Day, Crews, and Wilczynski 2001; Novejarque, Lanuza, and Martínez-García 2004; Kabelik, Weiss, and Moore 2008; LaDage et al. 2009; Cohen and Wade 2011). In lizards, the CX is associated with spatial memory processing (LaDage et al. 2009) and in mammals, GCs suppress hippocampal synaptic plasticity and promotes apoptosis (Tata and Anderson 2010). As these nuclei have GC receptors, they are highly susceptible to neuronal apoptosis, decreased synaptic connectivity and suppressed neural firing resulting in stress-related neuropathologies (Zhao et al. 2007; Rodrigues, LeDoux, and Sapolsky 2009; Reul et al. 2015). As the central regulator of the HPA axis, we predicted the HYP would show CORT differences following the hurricane, but this was not the case. However, forest lizards had higher CORT concentrations a month after, but this cannot be explained by negative feedback as forest anoles still retained HPA activity even after DEX administration. We also observed variation in the LFB after the hurricane, which was reversed within a month. Lesions of the LFB in *A. carolinensis* led to deficits in the challenge display (Greenberg, MacLean, and Ferguson 1979) but did not affect arousal and courtship (Greenberg, Chen, and Crews 1984). Increased behaviors

experienced in forest anoles following the hurricane may be related to CORT activation; however, this is far from understood. The fitness and behavioral implications of these differences are a source for future study, but we caution against assuming that increased CORT in the brain is always deleterious. This study demonstrated that although biological effects may not last long, their duration can vary between populations adapted to different conditions even on a microgeographic scale. Comparisons of urban and rural animal populations are not novel, but this is the first study to explore the intersection of urbanization and natural disasters, and to provide a framework for investigating how acclimation to one environment can prepare a species for another. One silver lining in the unfortunate reality of more frequent and severe hurricanes in the future will be further opportunities to test the urban resilience hypothesis.

## Supplementary data

Supplementary data are available at JUECOL online.

## Funding

This work was supported by the Division of Integrative Organismal Systems of the National Science Foundation under the RAPID program through NSF Award IOS-1762109 to H.B.F. Any opinions, findings and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect those of the National Science Foundation.

Conflict of interest statement. None declared.

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