Supplementary Materials S1

Corticosterone (CORT) Measurement

We validated the Corticosterone High Sensitivity EIA Kit (Immunodiagnostic Systems Ltd., Fountain Hills, AZ, USA) for use in *Anolis sagrei* by assessing both parallelism and quantitative recovery of a known CORT addition (spike).

Parallelism

To assess parallelism we generated a pooled plasma sample by combining plasma from both male and female *A. sagrei* (n=15). We serially diluted the pooled sample from 1:1 to 1:128 in assay buffer and assessed samples in duplicate. We compared the slope of the dilution curve to the standard curve, and the curves were not different (t_{10}=-0.368, p=0.720; Zar 1996). This indicates the kit measures CORT in *A. sagrei* in a fashion indistinguishable from the standards provided with the kit. The dilution curve was used to determine that a 1:10 dilution for samples was ideal as average samples would fall close to 50% binding values for the assay kit.

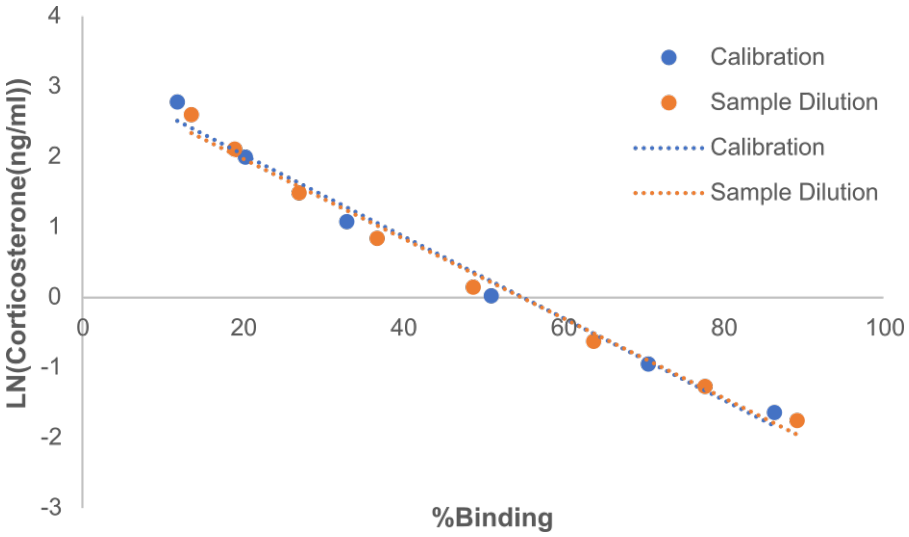

Figure S1. Comparison of slopes produced from serial dilution of control solution provided with kit (Calibration, blue) and from serial dilution of pooled sample (Sample Dilution, orange). Corticosterone concentrations were calculated by fitting absorbance values to a four point logistic curve. Equation for linear fit to calibration values: y = -0.0583x + 3.2043; R^2 = 0.9869. Equation for linear fit to pooled sample dilution values: y = -0.0567x + 3.1105; R^2 = 0.9882. Slopes were not significantly different indicating parallel responses were achieved.

Recovery

To assess recovery we generated a pooled plasma sample by combining plasma from both male and female *A. sagrei* (n=7) which was spiked with known concentrations of CORT from control standard provided with the kit. We created three dilutions of control solution from the kit (1:1, 1:5, and 1:10) to make a high, middle, and low spike. We split the pooled sample evenly into micro-centrifuge tubes and mixed with equal volumes of the diluted control standard to add known spikes of CORT to each sample as well as assaying a non-spiked sample of the pooled plasma. Each sample was assessed in duplicate.

CORT measured in the unspiked pooled sample was 15.01 ng/ml, and made up 8 ul (2.4016 ng) of each 50 ul spiked sample. Average recovery across the three spikes was 110.3% (range: 92.7-135%). The coefficient of variation in measured and calculated CORT for the three spiked samples was 4.55%.

Spike	Measured CORT (ng/ml)	Calculated CORT (ng/ml)	% Recovery
High	15.711	16.766	0.927
Medium	5.368	5.274	1.033
Low	4.341	3.838	1.350

Table S1. Values of CORT concentration measured from samples with high, medium, and low spikes of CORT added along with calculated concentrations and estimates of percent recovery.

References:

Zar JH. Biostatistical analysis. Englewood Cliffs: Prentice-Hall; c1996.

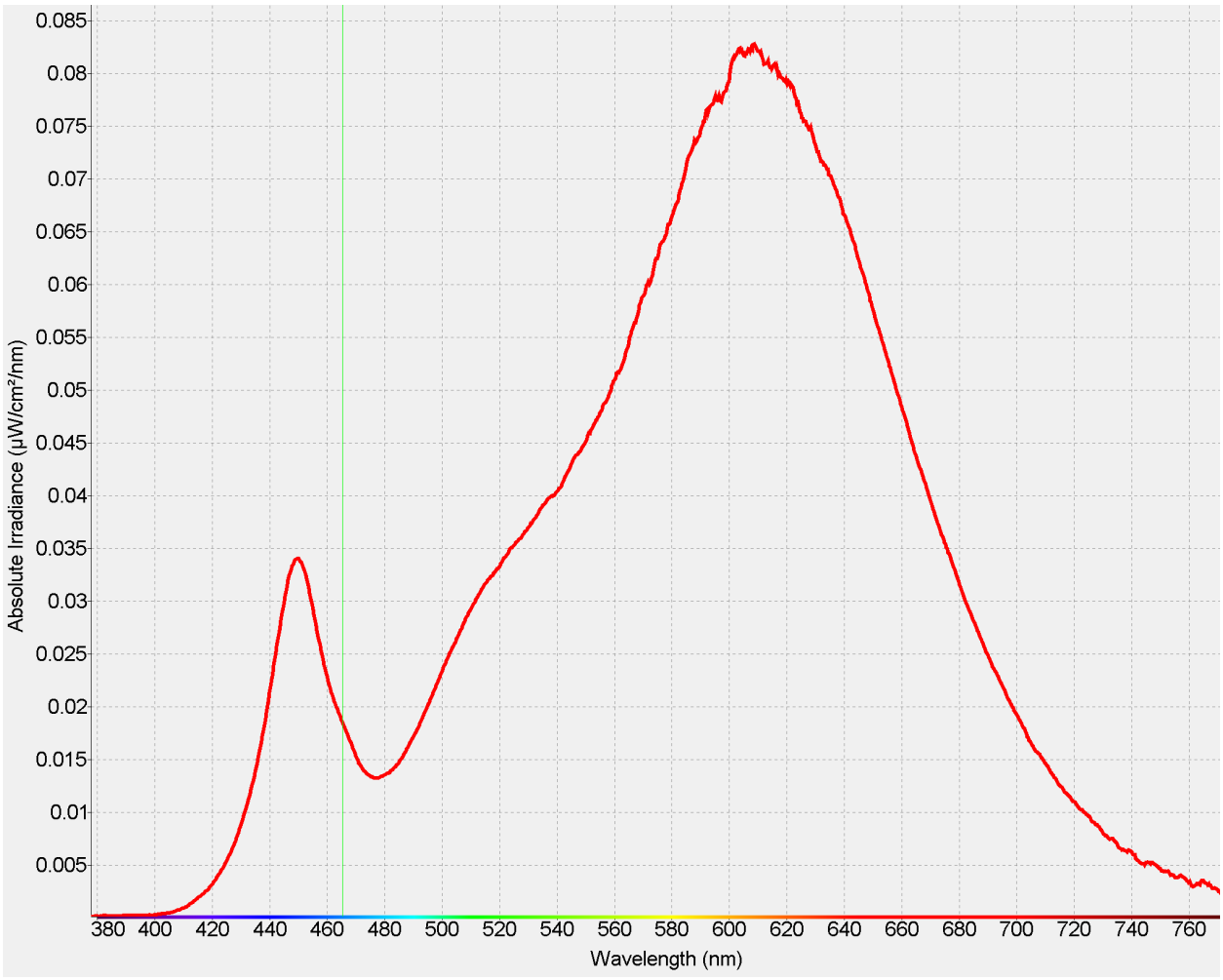

Figure S2. Absolute irradiance output spectrum of LED light source (Lighting EVER, 3000K) used to create ALAN treatment taken with cosine corrected probe on JAZ spectrometer (Ocean Optics).

Supplemental Materials:

S3: Scripts and Details for Analyses

Models used in conducting analyses with their R scripts. Starting models (for model selection) and final models noted where applicable, with model selection conducted via likelihood ratio testing. If no starting model noted, the *a priori* chosen best model was used in the final analysis.

Growth

We assessed impacts of ALAN on growth as change in SVL and body condition using linear models including ALAN treatment, sex, initial SVL, and initial body condition as predictors. Initial SVL was centered and scaled separately for each sex. Initial body condition was scaled. Two male lizards were excluded from analyses; one died during the experiment and another experienced high weight loss and was euthanized due to poor health.

Starting Model for Growth in SVL

```
SVLChangeStarting <-lm(SVLChange~ALAN*Sex+SVL*Sex+ BodyConditionResidual*Sex, data=gsc1)
```

Interactions that did not affect the response (as determined via likelihood ratio test) were excluded from final models.

Final Model for Growth in SVL

```
SVLChangeFinal<-lm(SVLChange~ALAN+Sex+SVL+BodyConditionResidual, data=gsc1)
```

Post Hoc testing for Growth in SVL

```
library(emmeans)

#Comparisons of all levels of ALAN*Sex (4)

SVLChangeFinal.em = emmeans(SVLChangeFinal, ~ Sex*ALAN)

cld(SVLChangeFinal.em)

pairs(SVLChangeFinal.em)

#Main effect of sex

SVLChangeFinal2.em = emmeans(SVLChangeFinal, ~ Sex)

cld(SVLChangeFinal2.em)

pairs(SVLChangeFinal2.em)
```

Body Condition:

Initial SVL was centered and scaled separately for each sex. Initial body condition was scaled.

Starting Model:

BodyConditionChangeStarting<-lm(BodyConditionChange~ALAN*Sex+SVL*Sex+BodyCondition*Sex, data=gsc1)

Final Model:

BodyConditionChangeFinal<-lm(BodyConditionChange~ALAN+Sex+SVL+BodyCondition*Sex, data=gsc1)

We tested for differences in body condition between male and female anoles at the start and end of the experiment using paired t-tests.

t.test(FemaleBCStart, FemaleBCEnd, paired = TRUE, alternative = "two.sided")

t.test(MaleBCStart, <MaleBCEnd, paired = TRUE, alternative = "two.sided")

CORT:

We assessed whether ALAN affected levels of CORT in plasma using a linear model with ALAN as a fixed factor and its interactions with sex, SVL, and body condition as a starting model. CORT was natural log-transformed, and SVL and body condition were scaled and centered separately for males and females.

Starting Model for CORT:

CORTstarting<-lm(LNCort~ALAN*Sex+ALAN*SVL+ ALAN*BodyConditionResidual, data=fmc)

Interactions that did not affect the response (as determined via likelihood ratio test) were excluded from final models.

Final Model for CORT:

CORTfinal<-lm(LNCort~ALAN+Sex+SVL+ BodyConditionResidual, data=fmc)

Onset of Reproduction:

We tested whether ALAN affected onset of reproduction in females (day of laying first egg) using survival analysis as implemented in the *survival* and *flexsurv* packages. Our starting model included ALAN and its interaction with initial SVL as well as initial body condition.

Starting Model:

LayDateStarting<-survreg(LayDate~ALAN*SVL+BCResid, data=ldt, dist="weibull")

The ALAN*SVL interaction that did not affect the response (as determined via likelihood ratio test) and was excluded from the final model.

Final Model:

LayDateFinal<-survreg(LayDate~ALAN+SVL+BCResid, data=ldt, dist="weibull")

Number of Eggs:

Model:

Initial SVL and initial body condition were used as covariates

NofEggs<-lm(NumberofEggs~ALAN*SVL+BodyCondition, data=ne)

Total Egg Mass:

Model:

Initial SVL and initial body condition were used as covariates

TotalEggMass<-lm(NumberofEggs~ALAN*SVL+BodyCondition, data=ne)

Proportion of Mothers Laying Eggs over the Course of the Experiment:

#####Basic statistics and chi-squared tests for proportions

#####Dark: 14/16 mothers laid eggs

#####Light: 16/16 mothers laid eggs

motherlayeggs<- matrix(c(16,0,14,2),ncol=2,byrow=TRUE)

colnames(motherlayeggs) <- c("Success","Fail")

rownames(motherlayeggs) <- c("Light","Dark")

motherlayeggs <- as.table(motherlayeggs)

motherlayeggs

#####Using monte carlo simulation to calculate p-values

chisq.test(motherlayeggs, simulate.p.value = T, B = 20000)

#####X-squared = 2.1333, df = NA, p-value = 0.4873

#####One-tailed test, p=.2436

Interval in Days between Eggs Laid:

We tested for effects of ALAN on the interval (in days) between eggs laid using a linear mixed model implemented in the *lme4* package with ALAN, individual egg mass, Julian Day, and SVL of mother as predictors, the interaction between mother's SVL and ALAN, and a random effect for mother. Interval between egg-laying was natural log-transformed. The first egg laid by each female was not included in

this analysis as interval could not be determined for these eggs. Models were run both with and without one outlier (interval = 26 days); results were qualitatively similar, and we retained the outlier in our final model.

Model:

IntervalModel<-lmer(log(Interval)~MotherSVL*ALAN+EggMass+JulianDate+(1|MomID), data=ei, REML=T)

Egg Morphology:

We tested for effects of ALAN on morphology of eggs using a set of models including ALAN, Julian Date, and SVL and body condition of mother as covariates.

Egg Wet Mass:

WetMassModel<-lmer(WetMass~ALAN+JulianDay+BodyConditionMother+SVLMother +(1|MotherID), data=wet, REML=T)

Egg Length:

LengthModel<-lmer(Length~ALAN+JulianDay+BodyConditionMother+SVLMother +(1|MotherID), data=wet, REML=T)

Egg Width:

EggWidthModel<-lmer(EggWidth~ALAN+JulianDay+BodyConditionMother+SVLMother +(1|MotherID), data=dem, REML=T)

Egg Dry Mass:

DryMassModel<-lmer(DryMass~ALAN+JulianDay+BodyConditionMother+SVLMother +(1|MotherID), data=dem, REML=T)

Egg Content Mass:

ContentMassModel<-lmer(ContentMass~ALAN+JulianDay+BodyConditionMother+SVLMother +(1|MotherID), data= dem, REML=T)

Egg Water Content:

WaterContentModel<-lmer(WaterContent~ALAN+JulianDay+BodyConditionMother+SVLMother +(1|MotherID), data= dem, REML=T)

Mother SVL When Producing First Egg:

We assessed whether SVL of females when producing their first egg differed with ALAN exposure

Model:

FirstEggSVL<-lm(MotherSVL~ALAN, data=dem1st)

First Eggs Produced by Mothers:

We assessed whether the first eggs produced by each mother differed in their morphology (wet mass, length, width, dry mass, or water content) with ALAN exposure of mother. These models used covariates that were significant predictors of each response variable in analyses using full datasets. For two females whose first clutches contained two eggs, measurements of the two eggs were averaged.

Egg Wet Mass:

FirstWetMass<-lm(wetMass~ALAN+MotherSVL, data=dem1st)

Egg Length:

FirstLength<-lm(Length~ALAN+MotherBodyCondition+JulianDate, data=dem1st)

Egg Width:

FirstWidth<-lm(Width~ALAN, data=dem1st)

Egg Dry Mass:

FirstDryMass<-lm(DryMass~ALAN+MotherBodyCondition, data=dem1st)

Egg Water Content:

FirstWaterContent<-lm(WaterContent~ALAN+MotherBodyCondition, data=dem1st)

Effects of ALAN on Egg Time to Hatching and Hatchling Morphology:

We assessed whether ALAN affected the incubation time of eggs to hatching, size, and body condition of hatchlings using linear mixed models with ALAN as a fixed factor, a random effect for mother, and egg mass, mother's SVL, and Julian day as covariates. Egg mass, mother's SVL, and Julian Day were centered and scaled.

Days to Hatch:

DaystoHatch<-lmer(Days~ALAN+MotherSVL+EggMass+JulianDate+(1|MomID), data=ham, REML=T)

Hatchling SVL:

HatchlingSVL<-lmer(SVL~ALAN+MotherSVL+EggMass+JulianDate+(1|MomID), data=ham, REML=T)

Hatchling Body Condition:

HatchlingBodyCondition <-lmer(BodyCondition~ALAN+MotherSVL+EggMass+JulianDate+(1|MomID), data=ham, REML=T)

Effect of ALAN on Proportion of Mothers with Eggs Hatching Successfully:

We assessed whether the proportion of mothers for which all eggs hatched successfully varied with ALAN exposure using a one-tailed chi-squared test with p-value determined via Monte Carlo simulation (20,000 replicates).

#####Creating table of mother success

mothersuccess <- matrix(c(8,0,7,4),ncol=2,byrow=TRUE)

colnames(mothersuccess) <- c("Success","Fail")

rownames(mothersuccess) <- c("Dark","Light")

mothersuccess <- as.table(mothersuccess)

mothersuccess

chisq.test(mothersuccess, simulate.p.value = T, B = 20000)

#####X-squared = 3.6848, df = NA, p-value = 0.1042

#####One-tailed test, p=.0526

Supplementary Materials S4:

Levels of ALAN intensity at perches of sleeping anoles at study site:

From 6/12/16 to 6/20/16 we measured light intensities at sleeping perches of anoles at our collection site using a TES 1332A Digital Lux Meter. Anoles commonly perched on branches, leaves, and fronds of vegetation including shrubs, cycads, and bushes. The habitat had a mostly closed canopy and no direct sources of ALAN. Light levels ranged from 0.0-0.1 lux (n = 104). Readings for only two anoles indicated a light level of 0.1 lux, with all other measurements indicating light levels below the detection limit of the meter (0.0).

Levels of ALAN intensity at perches of sleeping anoles at an urban park in Miami:

On 9/17/2016, we measured light intensities at sleeping perches of anoles in a park in the Miami metropolitan area (25.7460°, -80.2585°, WGS84) using a TES 1332A Digital Lux Meter. Habitats in the park included shrubs and small plants commonly used in landscaping, and, while multiple trees were present, the canopy was largely open. The park was illuminated by several streetlights which were not accessible to anoles for foraging on insects attracted to ALAN due to their height and distance from accessible perches. All measurements were taken at sites of anoles that were inactive and sleeping when encountered. Light levels (0.68 ± 0.23 lux; mean ± SE; n = 18) ranged from 0.1-3.8 lux with the lowest levels occurring at perches shaded from light by vegetation. All sleeping perches registered at least 0.1 lux whereas only 2% of sleeping perches at our natural collection site had light levels of ≥ 0.1 lux. We compared light levels at these sites using a Mann-Whitney U test, and light levels at the urban park were significantly higher than those at our collection site ($W = 1$, $p < 0.0001$).

Levels of ALAN intensity at perches exposed to landscape lighting in Miami:

In summer 2018, we measured light levels associated with a series of standard 150 W landscaping lights (GE floodlight 150W PAR38 halogen bulbs; n=8). These bulbs are commonly stocked at hardware and home improvement stores in the Miami area (where they were purchased) for landscape lighting applications and were mounted in weatherproof landscape light sockets. Light levels were measured using a TES 1332A Digital Lux Meter at distances of 2.5, 5, and 7.5 m from each light source at heights randomly assigned between 0.2 and 2 m. If a potential anole perch was within 0.25 m of the assigned location, we took data from the closest such perch. Many locations at which light levels were measured were blocked from direct exposure to the light source by intervening foliage, reducing light levels below maximal potential exposure levels. At each location, we measured light levels with the light meter perpendicular to the light source (representing maximal exposure) or at a 45° angle to the light source (representing a moderate level of exposure). Based on our experience, anoles using artificial lights to actively forage for insects attracted to lights generally do so no farther then 3-4 m from the light source, likely due to the lack of insects attracted to lights at distances >3-4 m from the source (see Fig. S4). Light levels varied due to the presence of foliage, but show that anoles close enough to landscape lights to benefit from foraging on insects attracted to the lights (<4 m), are likely to experience light levels comparable to or greater than levels to which anoles were exposed in our lab treatment (Fig. S3). Anoles are exposed to these levels of ALAN while foraging on insects attracted to lights in urban areas, including on walls of structures (Fig. S4A) and on natural perches and the ground near landscape lights (Fig. S4B and C) which are common in urban Miami (Fig. S4D). Measurements on landscape lights of the same

type (n=10) in 2014 yielded similar results, with a mean light level of 92 lux at a distance of 3 m from light sources (H. Moniz, unpubl. data).

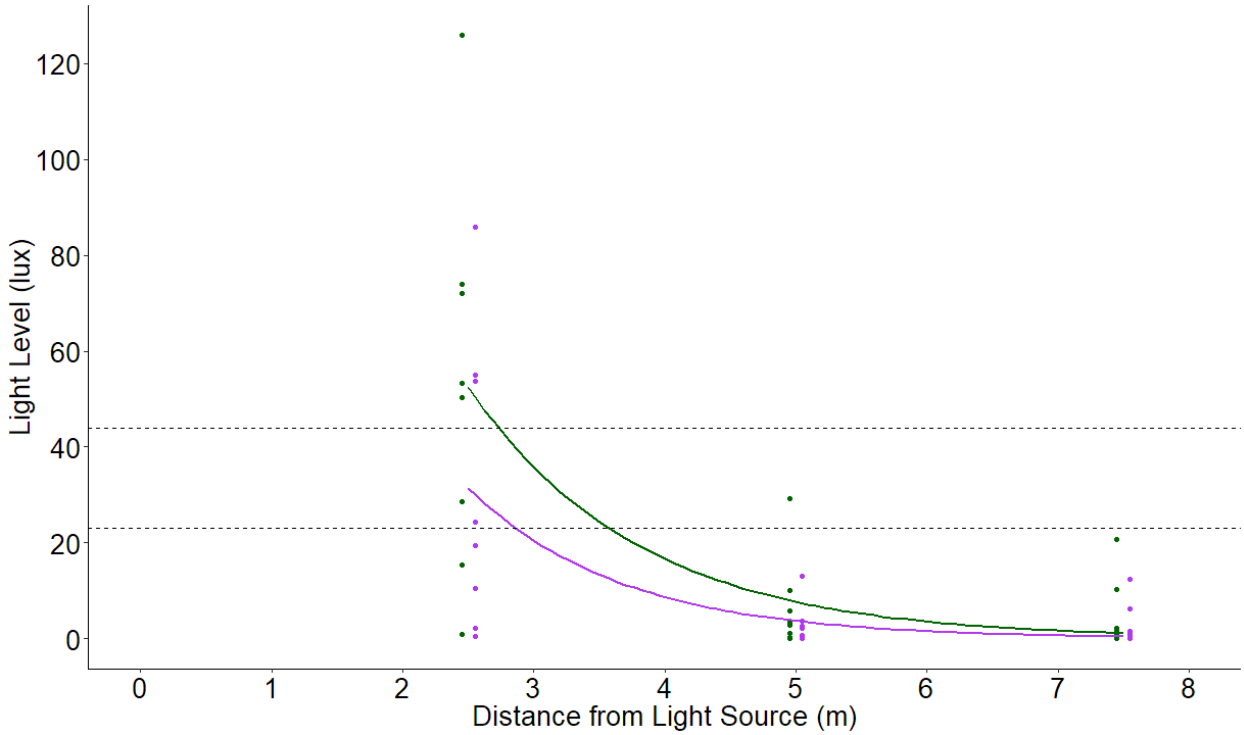

Figure S3. Light levels from perches in front of landscape lights at distances of 2.5, 5, and 7.5 m from light source with light meter held perpendicular to light source (maximal exposure, dark green) and at a 45° angle to light source (moderate exposure, purple). Light levels are offset on x-axis to improve readability. Dotted lines represent range of light levels in ALAN treatment in our lab experiment.

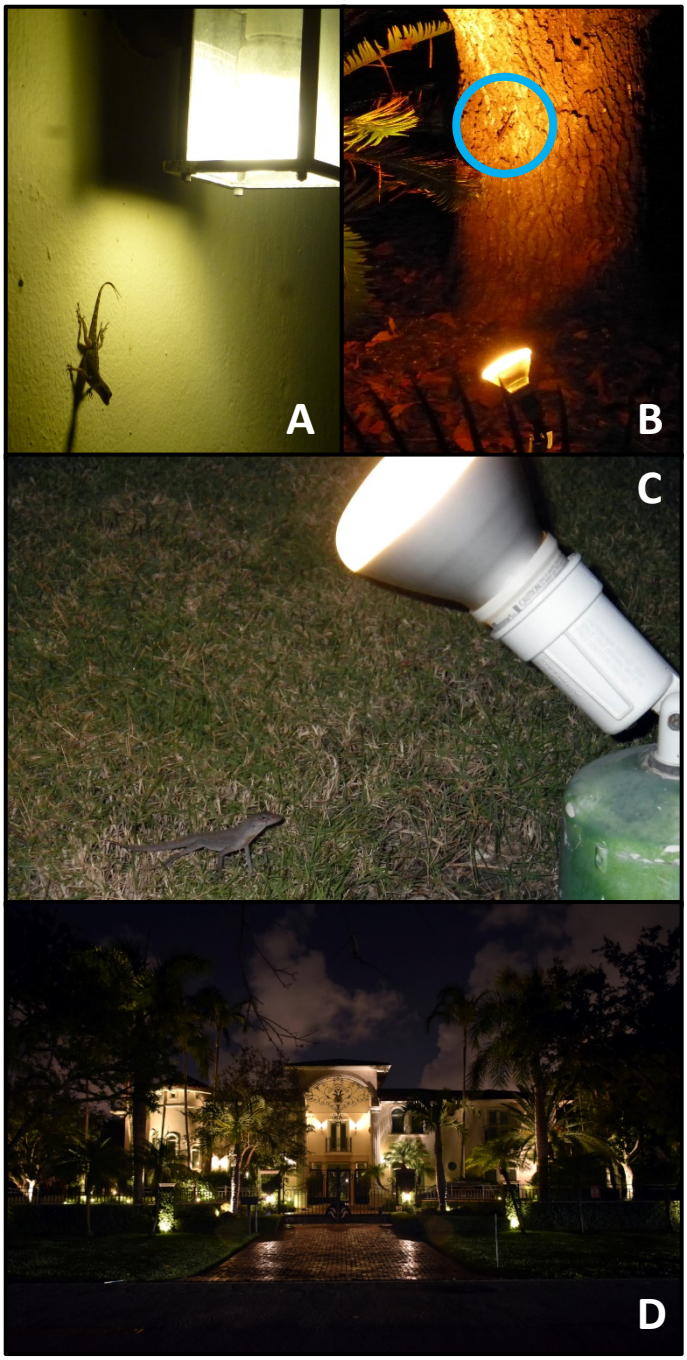

Figure S4. Anoles actively forage in close proximity to street, house, and landscape lights (0.25 m from light [A]; 1.25m from light [B]; 0.25 m from light [C]). Such lighting is common in residential areas near the collection site (<1 km) for anoles used in this experiment (D).