Color vision and signal color evolution in *Anolis* Lizards

By

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Abstract

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Anolis is a diverse genus of small arboreal lizards that utilize a visual communication system using a colorful expandable throat-fan (dewlap). They signal either to attract females or repel males. The environment the lizard lives in has influenced the coloring of these dewlaps. Species that occupy shaded habitats tend to have yellow dewlaps while those that inhabit brighter habitats tend to have red dewlaps. In experiment 1, we used a color perception assay to test whether a red or yellow stimulus against a green background was more visible to the lizard under different light intensities, mimicking a dewlap appearing in a forested or open habitat. Our results mirrored those predicted, suggesting that yellow is more visible under low light while red is more visible under high light. In experiment 2, we used a similar assay with different stimuli to test the relationship between dewlap and background brightness, independent of color, to see if dewlap brightness directly, influences detectability. The results weren't significant suggesting that anoles do not signal in a particular place in their environment and do not take in account the background brightness when signaling. In experiment 3, we wanted to test how males interacted with each other and how varying body temperature could affect the signaling of a lizard. The lizards with a high or optimal body temperature (lights on) had a higher aggression score than those lizards that had a low body temperature (lights off). Anoles with a low body temperature were lethargic resulting in a decrease in amount and frequency of signaling.

Introduction

Most animals use distinct signaling patterns for interspecfic and intraspecific communication. The mode in which these signals are relayed can come in many different forms including auditory, visual, tactile, and olfactory stimuli. Ants utilize the olfactory system where they produce a specific pheromone in order to leave a trail to different resources for other ants (Laidre and Johnstone 2013). Although ants use this to communicate to other ants about different locations, other animals can signal to attract the opposite sex, declare a territory or food source (i.e. repel others), strengthen pair bonding, or warn others about a predator. For instance, the vervet monkeys are an example of species that perform alarm calls to one another when a predator is approaching (Price et al 2015). *Anolis* lizards, utilize the visual communication via a throat-fan to attract females and repel males from a certain territory. Across different species this throat-fan, also known as a dewlap, can range in color, shape and size, but the reason why anoles display is fairly consistent (Fleishman 1992). Due to natural selection, the dewlap combined with other signaling patterns have evolved to benefit the anole in nature.

The anoline eye is designed for the animal to have a wide range of views. Because they tend to sit on perches and either wait for prey to capture or a conspecific to signal to, an anole needs to be able to see what is around them (Fleishman 1992). They rely heavily on a concept referred to as the “visual grasp” reflex where the peripheral retina sees something important, and therefore, transmits a signal causing the eye to shift towards this important item. The reason for response depends heavily on the motion they may detect in their periphery. This motion could be from their intended prey, a potential predator approaching, a possible female mate, or a near-by territorial male. Either way, the motion that is displayed must be visible to the receiver (Fleishman 1992). This is where the discovery of anoline color vision proves to be very important to the *Anolis* visual communication system. In order for their signaling to be effective, a fellow male or female anole must be able to see it. A recent study of 17 different species of *Anolis* lizards showed that anoles possess four classes of cone photoreceptors: with a long-wavelength-sensitive (LWS), a medium-wavelength-sensitive (MWS), a short-wavelength-sensitive (SWS), and an ultraviolet-sensitive visual pigment (UVS) (Loew et al 2002). Although the specific maximum absorbance varied from each species, they were all very similar. The LWS ranged from 560nm to 569nm with *Anolis carolinensis* as the outlier with a LWS at 625nm. The MWS ranged from 487nm to 503nm, the SWS ranged from 446nm to 467nm, and the UVS ranged from 364nm to 367nm (Loew et al 2002). The possession of these visual pigments translates to the ability to detect color differences and influences an anole's ability to signal to other anoles. The dewlap, which differs in color among different species, has the potential to communicate many things, but only if it is visible. Regardless of the specific function, the more strongly the dewlap color (or motion) stimulates the visual system, the more effective and efficient it is likely to be in carrying out the function. The fact that *Anolis* lizards possess some sort of color vision is crucial to the different behaviors and displays they perform.

Habitat preferences in anoles play an important role in the evolution of their visual communication system. Research has shown a correlation between dewlap color and natural habitat of a specific anole. The light conditions of certain environments have been thought to lead to the evolution of dewlap color. Although it was first thought that the contrast between the dewlap and habitat coloring determined dewlap-color evolution, Leal and Fleishman (2004) discovered a more important relationship: dewlap coloring and the radiance/irradiance ratio. Radiance is the light reflecting off a surface whereas irradiance is the light directly on a surface (Leal and Fleishman 2002). Bright dewlaps, like yellow and white, are more visible in dark habitats where the surrounding environment has low radiance, but the irradiance hitting the dewlap is relatively high. Dark dewlaps, like red and brown, are more visible in bright habitats where the surrounding environment has high radiance, but the irradiance hitting the dewlap is low relative to the radiance (Leal and Fleishman 2004). This contrast between different light habitats has influenced the evolution of where different *Anolis* lizards reside. A preliminary study looked at different West Indian Anoles and found a similar relationship between habitat type and dewlap color. Anoles living in the dark forest tended to have brighter dewlaps (white and yellow) whereas anoles living in an open environment had darker dewlaps (red and brown). The anoline displays must be seen by the intended receiver, and the lighting of where a specific anole displays influences this visibility (Fleishman 1992).

Although the pulsing of the dewlap is a very important part of an anole display, there are many other components to a display. The color of the dewlap pairs with rapid movements of head bobbing and four-legged push-ups to maximize the visibility of the intended receiver. This receiver could be far away as part of an “assertion” display or the receiver could be closer as part of a “challenge” display (Fleishman 1992). Depending on the situation the anole is in determines which display they produce and the function it will serve. The assertion display may attract female mates or repel other males from the territory. The challenge display is typically used when two territorial males come into contact (Fleishman 1992). These displays tend to be more intense and have higher costs, but the aggressiveness of a certain anole depends heavily on the resource holding power (RHP). Many different inputs can influence the RHP ranging from physical capabilities (like body size and temperature of body) to mental states (like time of year). These inputs have the potential to determine an individual's fighting ability, and consequently, the outcome of a fight (Garcia et al 2012).

In this study, we focused on three different, but related, aspects of the *Anolis* visual communication system: (1) the relationship between dewlap coloring and habitat preference (2) the impact of brightness contrast on signaling (3) the effects of altering the RHP on the anoline aggressive signaling. For the first experiment, we relied on the "visual grasp" reflex of anoles where the color perception assay utilized motion of potential dewlap colors (yellow and red). In addition to changing the colors, we also altered the lighting to resemble the change in habitat light availability. We predicted that in darker light conditions the yellow stimuli would be more visible and in brighter light conditions the red stimuli would be more visible. For the second experiment, we used the same assay as the first experiment relying still on motion and the "visual grasp" reflex, but used different stimuli. We wanted to see if dewlap/background brightness influenced detectability independent of color. We predicted that the stimuli that went from a higher brightness percentage to a lower brightness percentage would be more detectable. For the third experiment, we performed normal fighting encounters, and then changed variables (i.e. temperature of the anole body) to help assess the relationship between RHP and signaling behavior. If anoles “honestly” signal their RHP, we predicted that decreasing the temperature of the anole would consequently change the signaling behavior of that specific anole. However, if the function of signaling is only to allow each individual to assess physical attributes like size, then we do not predict a relationship between RHP and signaling behavior. These different components of the *Anolis* visual communication system will help us better understand why evolution has led to these certain behaviors in habitat choice and signaling.

Materials and Methods

Study Subjects

This study was carried out on male *Anolis sagrei* for both experiments. They were shipped from Florida and given a week to settle into their new environment before experiments started. Each lizard was individually housed in a small cage with a height of 24.5 cm, a length of 19cm, a back width of 10.5 cm, and a front width of 19.5 cm. In every cage, there was a horizontal perch placed 6 cm from the front and 7 cm above the bottom of the cage. The front of the cage was glass, and the other three sides of the cage were opaque white. Each cage had a 50-W light 9 cm in front of the cage and 11.5 cm above the top of the cage. When the anoles were not performing experiments, a combination of these lights and broad-spectrum fluorescent lights remained on. The video camera was positioned 36cm from the perch, and the stimulus was placed 26 cm away at an angle of 51° relative to the lizard’s eye staring directly out towards the camera (Figure 1, Figure 2). They were given water every day and fed worms every Monday and Wednesday and crickets on every Friday.

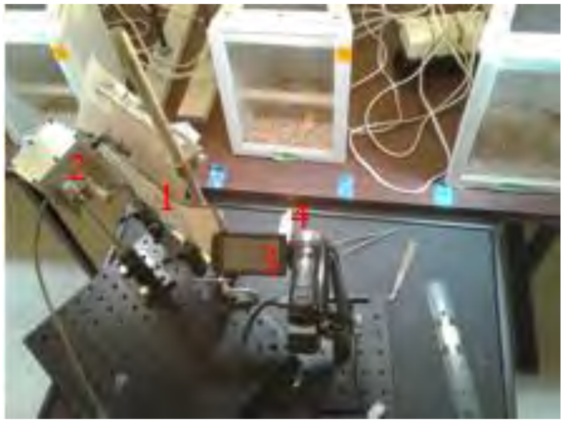

Figure 1. The overall setup of the experimental materials. 1 is the stimulus, 2 is the motor unit that changes the different stimuli, 3 is the camera, and 4 is the table in where everything is positioned.

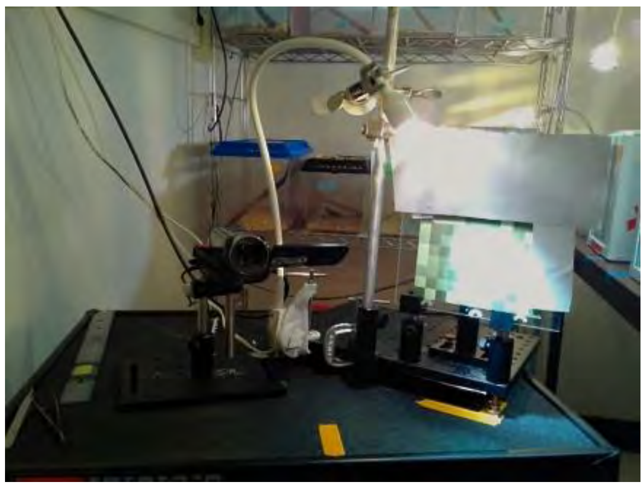

Figure 2. The front of the stimulus from the color perception assay where the smaller squares are composed of greens with different intensities. The 50-W light is positioned so the light is directly on the stimulus during the light condition.

Experiment 1: Color Perception Assay

For this experiment, the rectangular stimulus consisted of randomly placed small squares ranging in different intensities of green. The middle square was cut out in order to change the stimuli coloring (Figure 2). There were three different semicircle stimuli where each consisted of a green color with a certain intensity paired with either red, yellow or another green (with a different intensity) (Figure 3). The relative reflectance of each stimuli was recorded to quantify the different wavelengths of each color versus the percent reflectance, which is independent of light source (Figure 4). We also used the known spectral sensitivity of *Anolis sagrei* (see, e.g. Fleishman et al. 2016) to match the "brightness" (equals perceived intensity or luminance) as perceived by *A. sagrei* of each green to its associated color. For the control we intentionally introduced a 5% change difference in luminance from the initial to the final position.

Figure 3. The three stimuli used in the color perception assay. From left to right, it's the red, yellow, and green (control) semicircle stimuli. The yellow and red colors used had similar luminance to the green shared on each of the stimuli to eliminate intensity as a possible reason for a positive result.

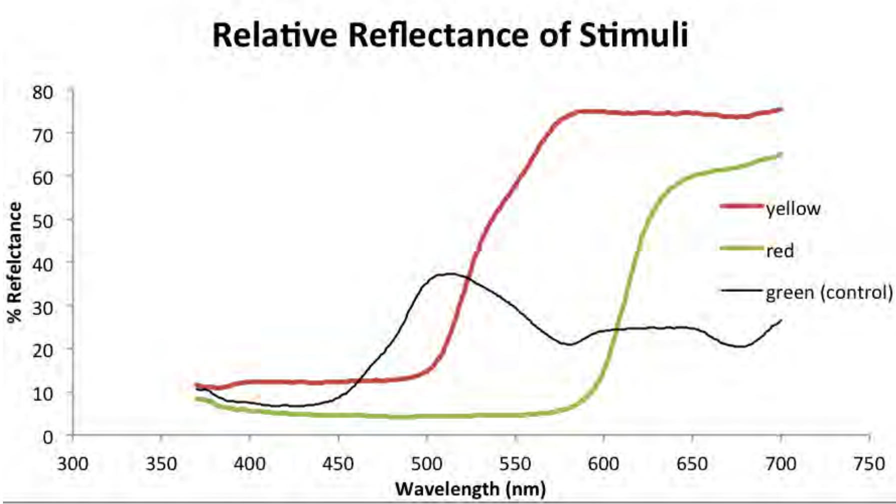

Figure 4. The relative reflectance of the yellow, red and green stimuli. We measured the color spectra for each of the colors so we could match them with a green with similar brightness values.

Each week a random order was assigned to determine which day would be the light or dark condition, but the lizards always had a 24-hour resting period in between the two conditions. The light environment consisted of all the 50-W lights above the cages to be on and an additional 50-W positioned directly in front of the stimulus field. Every 50-W light above the cages were turned off for the dark environment, and instead, two 50-W lights were positioned up towards the ceiling. However, for both light conditions the fluorescent lights were turned off. Every week, using a randomized order of the three different stimuli, each lizard performed the color perception assay under light and dark conditions. A positive response was noted when the lizard's beginning position (t1) changed to (t2) when he looked towards the stimulus within three seconds after the start button was pressed (Figure 5).

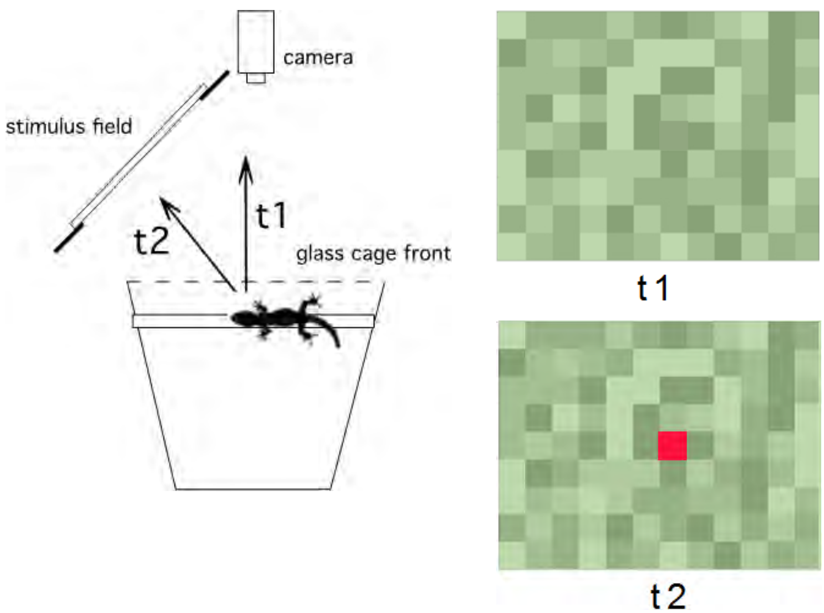

Figure 5. This was the general overview of the different features from the color perception assay. The lizard was positioned on the perch with a glass cage front. The camera was set 36cm away from the perch with the stimulus field next to it. A positive response was noted when the stimulus and the head or eye of the lizard changed from t1 to t2.

Experiment 2: Background Brightness Assay

This experiment utilized similar materials and methods to the color perception assay (figure 1, figure 5), but the stimulus and stimuli were different. The square stimulus comprised of smaller squares and the semicircle stimuli were on a gray-scale (figure 6). Based on the equation Y= -0.1032X + 7.4782, where Y is brightness and X is the percentage of reflectance, the 98 square stimulus was created using 16%, 30%, and 44% reflectance (similar to t1 in Figure 5). The five stimuli were determined using this same equation to keep a consistent 14% magnitude change between the brightness of both the stimulus and stimuli with a brightness range of 7.27 (2%) to 1.49 (58%). Stimulus 1 was a change from 30% to 30%, stimulus 2 was a change from 30% to 58%, stimulus 3 was a change from 30% to 2%, stimulus 4 was a change from 2% to 30%, and stimulus 5 was a change from 58% to 30%. This experiment was performed twice a week for each lizard where on the day of the experiment each lizard was tested with three randomly assigned semicircle stimuli. A positive response was noted when the lizard changed its head position from t1 to t2 (figure 5).

t1

Figure 6. This is the gray-scale stimulus field that the anole saw during his one-minute habituation period contrasted by the green-scale stimulus field seen in figure 5.

Experiment 3: Agonistic Behavior

In this experiment, six *Anolis sagrei* lizards were used. There were three 21.5 in X 13.5 in X 10.5 in (lengthXheightXwidth) cages with two lizards in each cage separated by an opaque gray divider. Only the front side of the cage was opened for viewing while the other three sides were covered with green construction paper to prevent the lizards in cages next to each other from interacting. There was a long wooden perch for every lizard, and four high intensity mercury vapor lights positioned 8 inches above each cage. These lights provided heat and broad-band illumination. For six weeks, this experiment was performed every Thursday morning alternating the lighting each week. The first week all four lights were kept on, and then the following week all the lights were turned off 12 hours before the trials. The third week cage 1 and 2 had the front light on with the back light off while cage 3 had the front light off and the back light on. The fourth week the opposite happened where cage 1 and 2 had the front light off and the back light on, and cage 3 had the front light on and the back light off. In week 5 all the lights were off (similar to week 2), and in week 6 all the lights were on (similar to week 1). During each trial a blue tarp was hung across the entirety of the room to minimize the human interaction with the lizards, and a hole was cut in the tarp for the video camera to sit. The grey filament blocking the two lizards in each cage was removed, and the record button on the video camera was pressed. The interactions between the two lizards were monitored and different signals including dewlap pulsing, head bobbing, two-leg or four-leg push-ups were noted. The fights were run for twenty minutes unless a lizard retreated three times from the other lizard. Once a fight was over, the two lizards were placed back on their respected sides, and the grey filament was returned. Analysis of the fights were based on a hypothetical aggression score determined by the scoring shown in Table 1.

Table 1. Point system used for the agonistic behavior experiment where each different signal was given a hypothetical number to help determine an overall aggression score for each lizard.

Signal	Score
Dewlap	1 point per dewlap
Headbobbing	1 point per sequence
Four-leg push up	2 points
Leap towards opponent	2 points
Retreat	-1 point

Results

Experiment 1

Positive responses were noted for the six different treatments – light control, dark control, light yellow, dark yellow, light red, dark red. The results of the individual lizards were analyzed within the larger group of eleven lizards by taking the average response proportions. For both the light and dark conditions for the control stimulus (green), there was little to no response. Although there was slightly more response to the control stimulus under the dark lighting, there was no statistically difference between either the bright or dark lighting. In the light condition, the response proportion was 0.145, whereas in the dark condition, the response proportion was 0.182. When comparing the response to the control, there were greater response proportions to both the yellow and red stimuli under the light and dark conditions. Within the yellow stimulus, the light and dark conditions showed no statistical difference. Under bright light, the lizards had a positive response proportion of 0.418. The dark condition produced a slight decrease in positive responses from the lizards where the proportion of positive responses was 0.4. The red stimulus had statistically significant results with a p-value of 0.02 when comparing the response rates under the light and dark conditions. The bright lighting had a greater proportion of positive responses at 0.509 whereas the dark condition had a proportion of positive responses of 0.327. There was a marginal significant difference between the yellow and red stimuli under the dark condition. A trend showed that when comparing the dark conditions, the yellow had a greater response rate than red (figure 7).

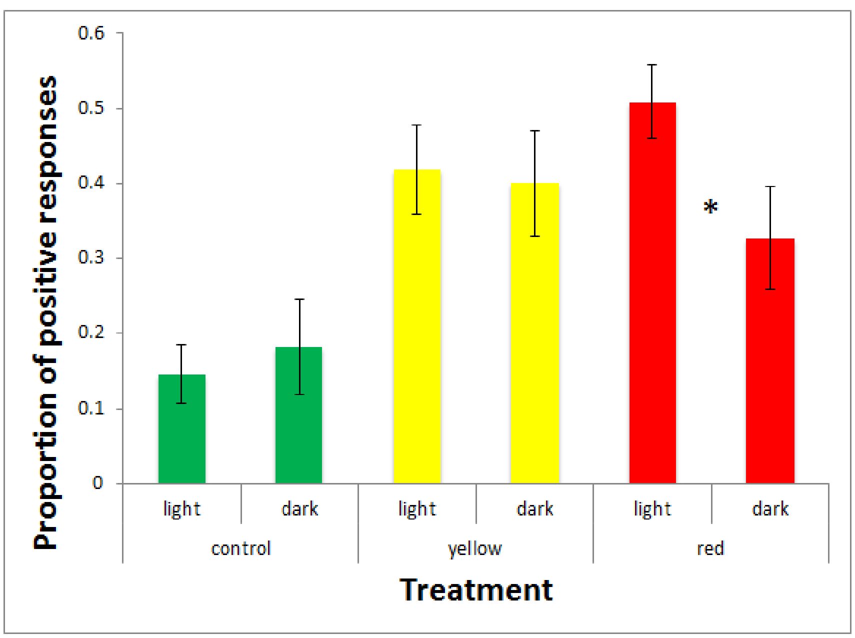

Figure 7. The proportion of positive responses of the lizards under the six different treatments in the color perception assay experiment. The bars represent standard error. The light control condition has a proportion of 0.145 ± 0.039, the dark control condition has a proportion of 0.182 ± 0.063, the light yellow

condition has a proportion of 0.418 ± 0.06, the dark yellow condition has a proportion of 0.4 ± 0.07, the light red condition has a proportion of 0.509 ± 0.049, and the dark red condition has a proportion of 0.327 ± 0.068. *The light and dark conditions for the red stimulus are statistically significant with a p-value of 0.02

Experiment 2

The positive responses were noted for the five different treatments – 30 to 30, 30 to 58, 30 to 2, 2 to 30, and 58 to 30. Similar to experiment 1, the results were analyzed in the larger group of ten lizards by taking the average response proportions. Lizard #10 was eventually disregarded in the results because he barely produced positive responses making him a dramatic outlier and causing the results to be skewed. Overall, there was no statistically significance between any of the treatments with a p-value of 0.22. The 58 to 30 brightness change produced the least amount of positive response proportion at 0.156 whereas the 30 to 58 brightness change produced the most amount of positive response with a proportion of 0.378. Both the 2 to 30 and 30 to 2 brightness change had the same amount of positive responses with proportions of 0.244. Finally, the control, or 30 to 30 brightness change, had the second lowest response rate with a positive response proportion at 0.2 (Figure 8).

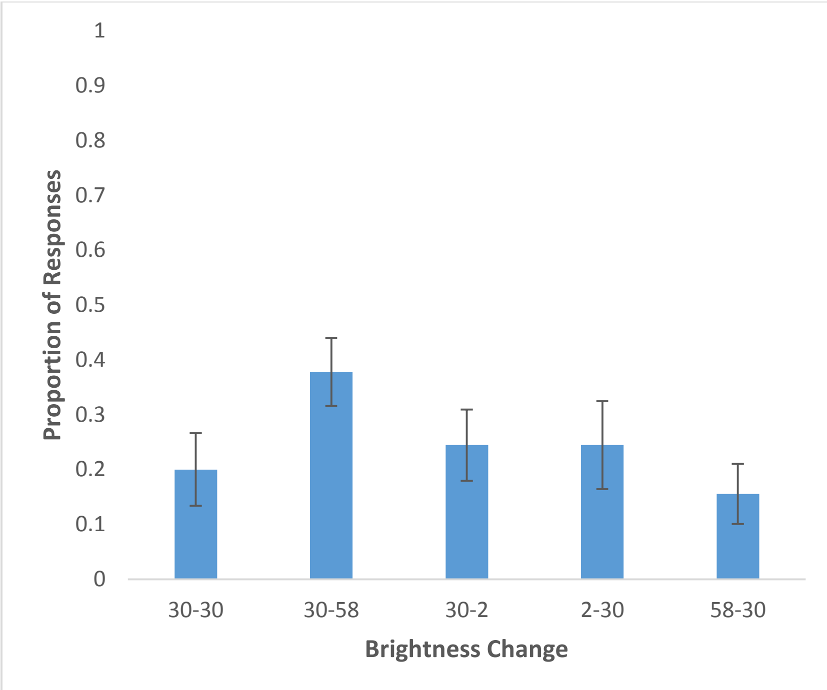

Figure 8. The positive response proportions of the five different treatments based on brightness changes in the background brightness assay. The bars represent standard error. The 30-30 brightness change has a proportion of 0.2 ± 0.066, the 30-58 brightness change has a proportion of 0.378 ± 0.062, the 30-2 brightness change has a proportion of 0.244 ± 0.065, the 2-30 brightness change has a proportion of 0.244 ± 0.08, and the 58-30 brightness change has a proportion of 0.156 ± 0.055. The p-value of this data is 0.22. *Note: Lizard #10 is not included in this data.*

Experiment 3

During week 1, the lights were kept on for both lizards in all three of the cages. The two lizards in cage #1 did not begin interacting until five minutes, but after that the interactions between the two escalated. Lizard 1 was the first to engage in fighting by moving to the other lizard's perch at 7:20 minutes. After the two exchanged some dewlap and head bobbing sequences, lizard 1 leaped towards lizard 2. The total fight lasted about 12 minutes where lizard 1 ended with an aggression score of 38 compared to 22, the aggression score of lizard 2. The next two cages had similar results, but both fights didn't last as long. Cage #2 had a fight that lasted five minutes where lizard 1 had a higher aggression score of 23 compared to lizard 2 that had an aggression score of

11. The two lizards in cage 3 had a fight last about 7:30 minutes. Lizard 2 signaled more resulting in an aggression score of 27. Lizard 1 did not signal as much, but still a fair amount, which resulted in an aggression score of 15 (Table 2). During week 6, the lights were kept on again for all cages. However, during week 4, a lizard in cage #2 passed away, and as a result the cage was no longer in the experiment because adding a new lizard would have messed up results. In cage #1, the fight lasted a total of 11 minutes. Lizard 2 was considered the “winner” with a higher aggression score of 33. Lizard 1 signaled, but ended up retreating three times. This lizard ended the fight with an aggression score of 16 (Table 7).

During weeks 2 and 5, all four lights were turned off. Overall, the lizards signaled less when they were under this condition compared to the lights on condition. The average aggression score over the course of all five lizard interactions (cage 1-3 during week 1, cage 1 and 2 during week 5) was 9.66 (Figure 9). The highest individual aggression score of one of these lizards was 18 compared the lowest individual aggression score of 5 (Table 3, Table 6). The fight duration was longer in these fights, and four out of the five fights did not end with a winner meaning neither lizard retreated three times within the 20-minute time limit. The one fight that did have a winner, both lizards had higher aggression scores compared to the other lizards in other fights. This fight lasted 15 minutes, which decreased the average fight duration for the lights off condition by a small amount to 19.2 minutes (Figure 10).

Weeks 3 and 4, the partial lighting condition, produced similar trends in the results. The average aggression score was 16.54, which is in the middle of the two other conditions (Figure 9). However, the individual aggression scores of the lizards that had the light on had an average of 22.4 compared to 9.8, the average of the aggression scores for the lizards that had the light off. The duration of these fights had a similar average to the lights off condition with an average of 18.67 (Figure 10). However, unlike majority of the lights off lizard fights, all five of these fights ended with “winners” (Table 4, Table 5).

Table 2. Lights on, week 1 individual lizard results.

Cage #	**1**		**2**		**3**	
Lizard #	**1**	2	**1**	2	1	**2**
Aggression Score	38	22	23	11	15	27
Duration (minutes)	12		5		7:30	

Note: Winner of each fight is indicated by the red, underlines.

Table 3. Lights off, week 2 individual lizard results.

Cage #	**1**		**2**		**3**	
Lizard #	1	2	1	2	1	**2**
Aggression Score	11	15	5	7	12	18
Duration (minutes)	20		20		15	

Note: Winner of each fight is indicated by the red, underlines.

Table 4. Partial lighting (lizard #1 in cages 1 and 2 had the light on; lizard #2 in cage 3 had the light on), week 3 individual lizard results.

Cage #	**1**		**2**		**3**	
Lizard #	**1**	2	**1**	2	1	**2**

Aggression Score	26	12	15	4	9	20
Duration (minutes)	17		18		17	

Note: Winner of each fight is indicated by the red, underlines.

Table 5. Partial lighting (lizard #2 in cage 1 had the light on; lizard #1 in cage 3 had the light on), week 4 individual lizard results.

Cage #	**1**		**3**	
Lizard #	1	<u>2</u>	<u>1</u>	2
Aggression Score	16	27	24	8
Duration (minutes)	20		20	

Note: Winner of each fight is indicated by the red, underlines.

Table 6. Lights off, week 5 individual lizard results.

Cage #	**1**		**3**	
Lizard #	1	2	1	2
Aggression Score	7	9	11	5
Duration (minutes)	20		20	

Note: There were no winners in these fights.

Table 7. Lights on, week 6 individual lizard results.

Cage #	**1**		**3**	
Lizard #	1	<u>2</u>	<u>1</u>	2

Aggression Score	16	33	24	13
Duration (minutes)	11		6:30	

Note: Winner of each fight is indicated by the red, underlines.

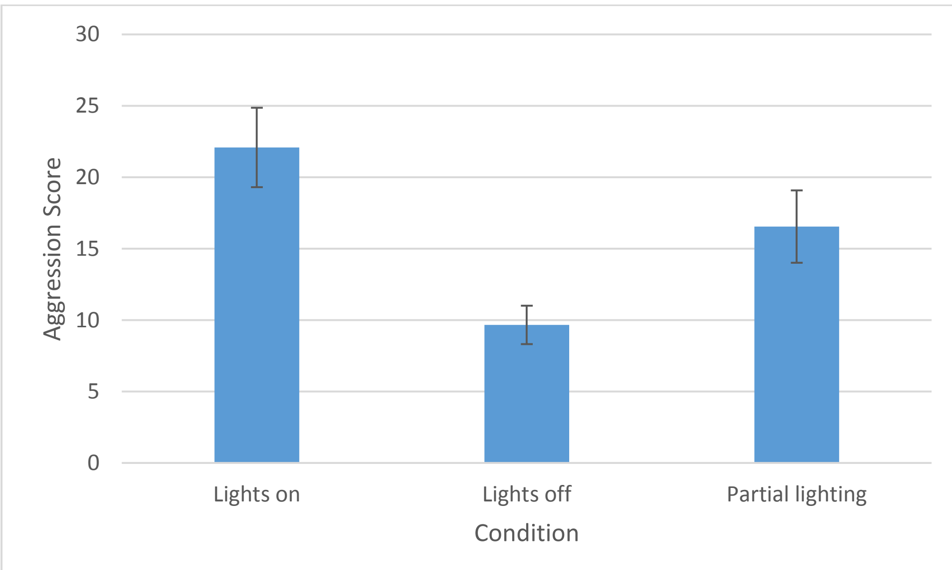

Figure 9. The average aggression scores of the lizards in five different fights (n = 6). The bars represent the standard error. The lights on condition has the highest average aggression score at 22.08 ± 2.78 compared to the lights off condition, which has the lowest average aggression score at 9.67 ± 1.35. The partial lighting condition has an average aggression score at 16.54 ± 2.53.

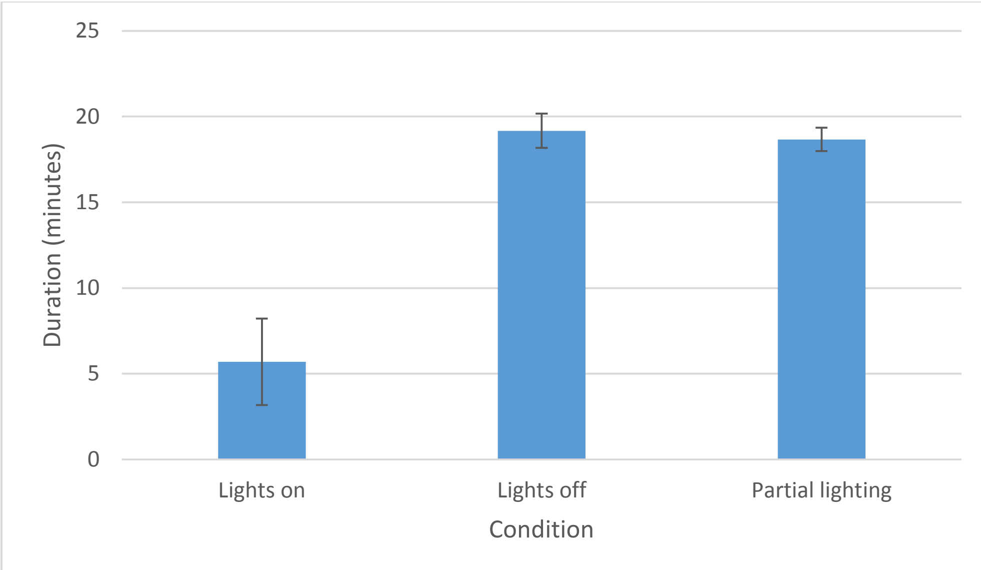

Figure 10. The average fight duration of the lizards in five different fights (n = 6). The bars represent the standard error. The lights on condition has the shortest fight duration at 5.7 ± 2.52 minutes. The lights off and partial lighting conditions have similar fight duration averages at 19.2 ± 1 and 18.67 ± 0.678, respectively.

Discussion

In experiment 1, we found that the different light conditions had a strong effect on the visibility of the yellow, red, and green stimuli. There was little response to the control, or green to green stimuli, which was expected in the hypothesis. Considering the lizards responded positively to both the yellow and red stimuli compared to the green shows that the stimuli effectively represented dewlaps flashing in nature. The consistent response to the yellow stimuli supports the color modelling theory where as the brightness decreases the distance between green and yellow in perceptual color space stays relatively the same. Because the distance is not changing, the response rate for the yellow stimulus under both the light and dark conditions is not statistically significant. These results could explain why research has shown that yellow and white dewlaps have a greater prevalence, especially in darker habitats (Fleishman et al 2009; Macedonia et al 2014). If the lizards have a fairly equal response rate to the yellow stimulus under both the light and dark conditions, we should see yellow dewlaps in both bright/open and dark/shady habitats. However, in the same studies, they found red and orange dewlaps were the most detectable in all habitats (Fleishman et al 2009; Macedonia et al 2014). The same color modelling theory used for the yellow stimulus results can be applied to the red stimulus results. However, for the color red, as the brightness decreases, the distance between red and green in perceptual color space becomes smaller. The decrease in distance causes the red stimulus to be less discriminable against the green background due to the reduced total photon flux of the red under dark lighting. This explains why the lizards had a significantly greater response rate to the red stimulus under the light condition compared to the dark condition. Overall the results supported our hypothesis, the red stimulus, representing a red dewlap, was more visible in bright, open habitats, and although there was no difference between the different light conditions, the yellow stimulus, representing a yellow dewlap, was more visible in darker habitats because it reflects more light.

A similar experiment was performed by Fleishman and Persons where they wanted to assess an anoles ability to distinguish brightness contrast between different color stimulus/background combinations. However, in this experiment the background was a uniform color instead of having the background composed of various brightness green-scaled squares. They also varied the brightness contrast between the stimuli and background whereas we kept each contrast difference between the two colors on each stimulus pair consistent. When comparing the brightness contrast of 0 for the stimulus/background combinations – green/green, yellow/green, orange/green, red/green – the orange and red had a higher response probability than green and yellow and had a similar response probability to each other (Fleishman and Persons 2001). This data supports our data in terms of the color visibility when brightness differences are not being accounted for; in our data, red was more visible than the yellow, and both red and yellow were more visible than green. Overall, red/orange and yellow dewlaps seem to be efficient in signaling against the green vegetation in nature.

In experiment 2, we found no statistical significance between any of the brightness stimuli. The only brightness change that had marginal significance when compared to the other stimuli was the 30% to 58% change. Although there was no significance between the different stimuli, the results show that the lizards did not notice the change in brightness of the center square. They took in account the overall brightness of the stimulus field causing them not to see the change in brightness of the one singular center square regardless of if it was changing from a lighter percentage to a darker percentage or vice versa. This is important because it shows that in nature it doesn't matter where the lizard displays his dewlap signal. If there were statistical results, the experimental setup was designed for positive responses to indicate that the lizards noticed a switch in the center square because they took the average brightness of the eight gray-scaled squares around the one center square. However, our results show that the brightness surrounding the actual dewlap does not matter, but more importantly, the overall brightness of the environment the lizard is signaling in seems to matter more.

In experiment 3, the hypothesis seemed to be supported by our results. When the lights were on, the anoles were eager to engage in signaling and fighting, but when the lights were off, majority of the fights lasted the whole 20 minutes with limited signaling. For the partial lighting fights, all the lizards that had the light on “won” each interaction where their aggression scores resembled those of the lights on. The losers of these partial lighting fights had aggression scores similar to the lights off lizards. This data shows that when one lizard had the resource to produce an optimal body temperature, the other lizard without the resource did not try to match his signaling. *Anolis sagrei*, like all anoles, live in warm climates and need the warmth in order to perform to their best capability. For the lizards that did not have the lights available to them 24 hours before the fight took place, as seen in both the partial lighting and lights off lizards, produced much lower aggression scores to those that had the lights available to them. These lights were to provide the warmth the lizards needed to obtain the optimal body temperature. When the lizards did not have the optimal body temperature (lights off lizards), they did not perform as well and the fights lasted longer. The lizards were lethargic, and therefore, signaled less. Overall, the anoles agonistic behavior (i.e. signaling and aggression scores) seemed to be very affected by the changes in temperature. This alteration in physical capability decreased an anole’s willingness to engage in territorial signaling.

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