6-2016

# The Use of A Feeding Assay to Test the Effects of Visual Color Contrast on Stimulus Visibility in the Lizard Anolis sagrei and the Evolution of Anolis species in the Dominican Republic

Austin Burnham
*Union College - Schenectady, NY*

# The use of a feeding assay to test the effects of visual color contrast on stimulus visibility in the lizard *Anolis sagrei* and the evolution of *Anolis* species in the Dominican Republic

By Austin Burnham
Advisor: Dr. Leo Fleishman
Dept. of Biology, Union College, Schenectady, NY 12308

**Abstract:** Many animals depend on color signals for communication. It has been hypothesized that signals evolve toward maximum visibility against natural backgrounds. Testing this hypothesis requires a way to assess the influence of signal/background color contrast on signal visibility. Most tests such as these rely on training, which can be very time consuming and difficult for some types of animals. We created a method for testing color contrasts using the lizard *Anolis sagrei*. We colored dead worms using powdered food coloring and placed them on backgrounds of varying colors and luminance. We presented stimuli in pairs that differed in contrast with the background, and determined that most individuals would choose the more visible food item first. We examined the effects of luminance and color contrast on stimulus visibility, and also tested for any inherent bias in preference for specific colors. Finally, we tested the effects of low light conditions on spectral contrast based visibility. We expected low light to reduce the effects of spectral contrast because it is known to elevate color discrimination thresholds. We found that this method is an effective approach to testing signal visibility in an animal that is difficult to study with standard conditioning techniques. We also wanted to see if dewlaps evolved towards maximum visibility in *Anolis* species in the Dominican Republic. We collected light habitat data, dewlap color data, and body color data on five different species of anoles in the Dominican Republic and compared the data using a tetrahedral perceptual color space program. We found that not only did the background play a role in the evolution of the dewlap color, but also species discrimination was a driving evolutionary factor in *Anolis* species of the Dominican Republic.

**Introduction:** Many animals depend on color signals for communication. One group of animals that relies heavily on color signals for communication in the wild is the genus *Anolis*. Male anoles use a colorful throat fan, called the dewlap to communicate. There are a variety of colors throughout the different *Anolis* species and they will flash their dewlaps for courtship, notifying the presence of a territory holder, and warning off predators by using their dewlap as a deterrent (Losos 2009). They are able to perceive different colors through the use of photosensitive pigments in their eyes called cones. It is believed that they have no rods, but four spectral classes of cones. These include short wavelength sensitive (SWS) cones, medium wavelength sensitive (MWS) cones, long wavelength sensitive (LWS) cones, and ultraviolet sensitive (UVS) cones (Losos 2009). The responsiveness of a cone depends on the intensity or number of photons per second of the source and on the wavelength of the photons. The ratio of the responses of the different classes of cones results in color perception (Bradbury and Vehrencamp 1998).

The appearance of colors in their environment can vary in a variety of ways. Spectral content is one way colors can vary, varying by spectrum or wavelength composition. Colors can also vary by brightness. Brightness refers to the overall perceived intensity of light emitted or reflected from a surface. In some cases the term brightness has been used incorrectly to refer to color saturation. Therefore, it is better to use the term luminance in order to describe the perceived intensity of light emitted or reflected from an object (Kemp et al. 2015). Earlier studies have shown that a large spectral contrast between an object and its environment increases the likelihood that an anole will detect the object. However, when there is a large difference in luminance between an object and a background, there is very little change in the responsiveness of the lizards (Fleishman & Persons 2001). Fleishman et al. (2009) showed differences in luminance in dewlaps do not affect visibility because the natural background is variable and on

average the contrast ends up being the same for different species.  They showed that the transmitted light does not increase luminance contrast between the background and the dewlap. Based on earlier studies, we hypothesized that color contrast would play more of a role in distinguishing between the food and background than luminance contrast.

We wanted to study the role that color versus background contrast played in making the anoline dewlap more visible to conspecifics.  In particular, we wanted to study the relative roles of luminance and color contrast in object discrimination. Finally, we wanted to test whether increased overall illumination would make colors easier to distinguish for anoles. We did this by creating a feeding assay using *Anolis sagrei*, where the lizards would discriminate different colored worms on backgrounds of varying color and luminance. We also wanted to create an approach that was cost effective and required little time since anoles are difficult to study using standard conditioning techniques.

Many animals rely on visual signals in the wild and signal visibility is very important (Bradbury and Vehrencamp 1998). It is hypothesized that visual signals evolve for maximum visibility against their natural background (Bradbury and Vehrencamp 1998). Male anoles use their dewlap as a visual signal and there are a variety of different colored dewlaps across the dewlap species, even anoles with UV color in their dewlap (Fleishman et al. 1993). It has been suggested that dewlap color evolved in the different species not only for maximum visibility against the background, but for discrimination between the different species as well (Fleishman 2000). This has idea has been supported in studies of anoles in Puerto Rico, as well as Jamaica (Losos 2009, Fleishman et al. 2009). We wanted to see if this was true as well for *Anolis* species in the Dominican Republic. We measured the light habitat, dewlap color and body color for several Dominican *Anolis* species to see if they showed the same trend in their evolution.

**Methods:** In order to test for the perception of color contrast in *Anolis sagrei*, we used dead worms as stimuli. We first had to train the lizards to eat dead worms and we did this by presenting each lizard with a live worm on a plastic dish. As soon as they had eaten it, we placed a dead worm on the dish. We killed the worms by either storing them in the freezer or by removing their heads. When we felt that a lizard was well-trained to eat dead worms, we needed to train it to eat dead worms that were colored with food powder. We used food coloring powder from Sweet XDreams Fun Confections to paint the worms and we used the colors avocado green and pink for our experiments. These colors also matched in *Anolis sagrei* luminance. We used paint brushes to paint the food coloring powder onto the worms and although both these colors came from the same company, pink was harder to paint onto the worms due to it being a much finer powder. To get the powder to stick to the dead worms we would kill them live by removing their heads and using the bodily fluid, we spread it over the body that worked as a natural adhesive for the pink food powder to stick onto.

Once the lizards had become accustomed to eating the dead colored worms, we began our color contrast experiments. The behavioral experiments consisted of three different experiments. In the first experiment, we would place an avocado green worm and a pink worm on a background that matched one of the worms. We used calibrated Munsell colored paper that matched in spectrum and luminance to one of the colored worms. We performed this scenario using first pink Munsell paper and then avocado green Munsell paper, which were placed in plastic dishes. A thin plastic layer was added to prevent the staining of the colored paper by the food coloring powder and the two worms of different colors were placed in the dish so that they

would not be touching. Finally, the worms were placed in different orientations with respect to the lizard so that no bias was formed.

The second experiment was very similar to the first experiment. It only varied in the type of calibrated Munsell paper that was used. We still used Munsell paper that matched the worms color spectrum (avocado green and pink), but we chose paper that had a luminance contrast that was lighter than the worms. We again performed this experiment two times, one with the lighter pink background and one with the lighter avocado green background.

The third experiment was the same as the first experiment except it took place in low light conditions in order to test their spectral contrast based visibility. For all three experiments we used a trial size of twelve lizards.

Field study

We collected the data on five different species of anoles, *Anolis brevirostris*, *A. coelestinus*, *A. cybotes*, *A. distichus*, and *A. olssoni* in the Dominican Republic. We collected data on light habitats, dewlaps colors, and body colors for a 10-day period in July 2015 in the province of Barahona, which is located in the southwest of the Dominican Republic. To measure light habitats, we first had to locate individuals of each species in the forest. Once we spotted a lizard, we observed it until it displayed or ten minutes passed. We then moved directly to the location where the lizard was observed and measured the light habitat using an Ocean Optics JAZ spectrometer with an attached fiber optic cable with a radiance lens, which measures a two degree circle. We measured the radiance towards the right and left and irradiance towards right and left. To measure radiance we pointed the radiance lens parallel to the ground. To measure

irradiance we pointed the radiance lens at a diffuse white reflectance standard that was also parallel to the ground, which collects the light from a full hemisphere normal to a surface.

In order to measure the dewlap and body colors of different anole species, we needed to collect many individuals from the different species. We used a modified Cabela's Panfish Pole which had a noose that was made out of dental floss or fishing line and would loop it over their head. We would then quickly pull back to secure the loop and store the lizards so that they could be brought back to the hotel for color measurements. The collection process did no harm to the lizards and all the lizards were released back to the general location where they were captured.

The set-up we used to measure the dewlap colors and body colors consisted of an Ocean Optics JAZ spectrometer with a fiber optics cable that had a flash pulsed Xeon lamp at the end. Lizards were held in a special apparatus with surgical tape. The tip of the dewlap was grabbed with a pair of fine forceps with opening controlled by a set-screw. This was mounted on a modified microscope stage that allowed us to gently pull the dewlap open to its natural extent. The tip of a fiber optic cable was positioned 2 mm from the surface of the dewlap. Light from a pulsed-xenon light source was delivered to the front of the dewlap. For reflectance measurements light was passed to the dewlap through a ring of six optical fibers, and recorded from a single fiber at the center of the apparatus. For transmission measurements light was delivered by the same ring of fibers but was recorded from a single fiber positioned 2 mm from the opposite side of the dewlap. We measured the reflectance and the transmission of the center of the dewlap and the edge of the dewlap, but only the reflectance of the body. For the dewlaps that had more than one color area, we measured both colors. For the species *A. coelestinus* the dewlap color was uniform so we only measured one region. Each reflectance or transmittance was divided by the reflectance of a calibrated white diffuse reflectance standard. For more

details about the procedure for field data collection of the light habitats, dewlap colors, and body colors of anole species, see Fleishman et al. (2015) and Fleishman et al. (2009), which has similar procedures. With regards to the actual analysis of the background data, dewlap color data, and the body color data, we used a computer program that consisted of a 3D model of a tetrahedron. The tetrahedron is a representation of the color space of a model anole with each colored vertices representing the four cones. In the figures in this paper the blue vertex represents the SWS cone, the green vertex represents the MWS cone, the red vertex represents the LWS cone and the purple vertex represents the UVS cone. Then for each color point the relative stimulation of UVS, SWS, MWS and LWS cones is calculated and each point is plotted as a distance from each of the tetrahedron vertices.

**Results:**

**Experiment 1**

In experiment 1 we placed two colored worms, avocado green and pink on a background that matched one of the worms with regards to color spectra and luminance. We found that the lizards would choose the worm that didn't match the background more often than not. This data can be seen in Table 1 and it can be seen graphically in Figure 1.

Table 1: Color discrimination responses of *A. sagrei* for experiment 1

| No match response | Match response |
|---|---|
| 14 | 2 |

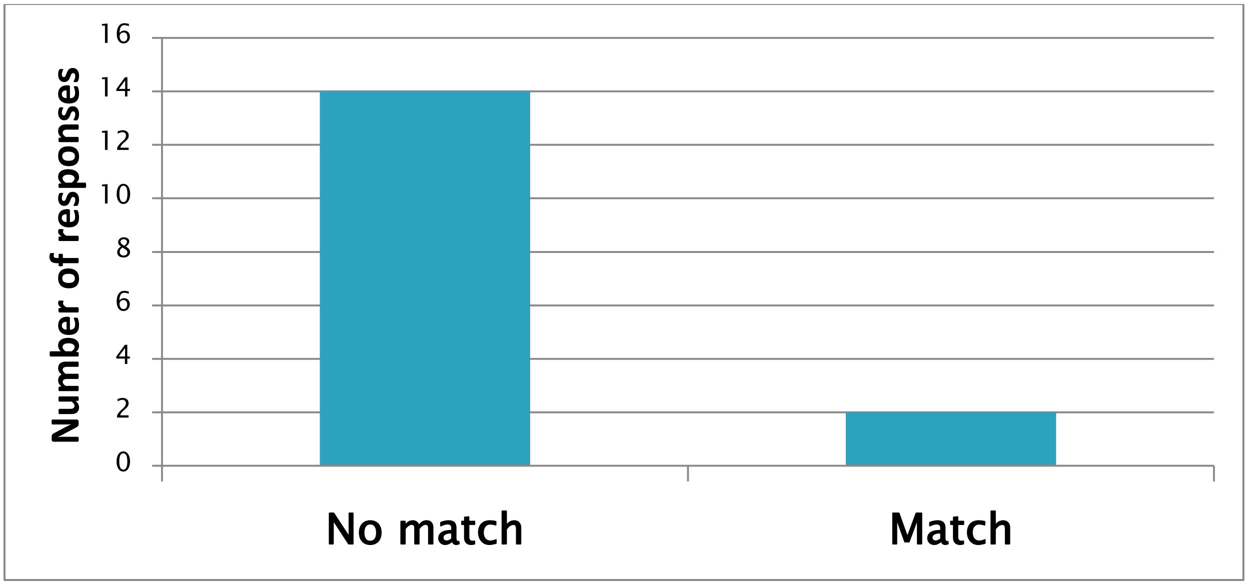


Figure 1: Data from Table 1 shown graphically and N = 16 total responses from *A. sagrei*. There is a significant difference between the two different responses with the no match response having a greater number of responses (P<0.05, binomial test).

**Experiment 2**

In experiment 2 we again placed two different colored worms, avocado green and pink, on a background that matched one of the worms with regards to color spectra. However, there was a luminance contrast with the background where the background was lighter than the worms. Although we found that the lizards would choose the worm that matched the background more often than not, this was not a significant difference (P>0.05, binomial test). This data can be seen in Table 2 and it can be seen graphically in Figure 2.

Table 2: Color discrimination responses of *A. sagrei* for experiment 2

| No match response | Match response |
|---|---|
| 6 | 10 |

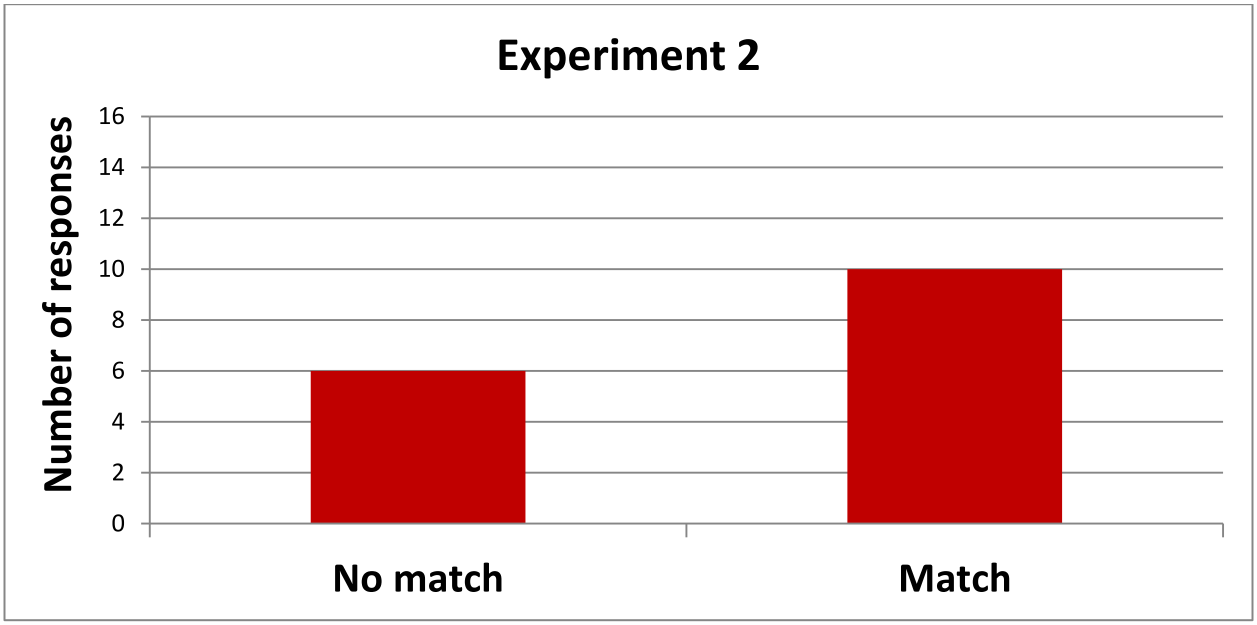


Figure 2: Data from Table 2 shown graphically and N = 16 total responses from *A. sagrei*. There is a little difference between the two different responses with the match response having a slightly greater number of responses.

**Experiment 3**

In experiment 3 we used the same conditions as in experiment 1, but under low light conditions. We were unable to collect data due to the lizards unwilling to eat the food under the low light conditions.

## Field Data

We collected data on five different species of anoles, *Anolis brevirostris*, *A. coelestinus*, *A. cybotes*, *A. distichus*, and *A. olssoni*. We collected the light habitats, dewlaps colors, and body colors of each species. The data is presented by each individual species. A comparison of the light habitats between the species and the dewlap colors is then presented.

### *Anolis brevirostris*

A picture of *A. brevirostris* can be seen in Figure 3.

Figure 3: An *Anolis brevirostris* specimen with dewlap extended.

There were 34 light habitats measured overall for this species and there is a large peak in both spectra between 530-570 nm. The average spectra of the radiance and irradiance of their light habitat can be seen in Figures 4 and 5.

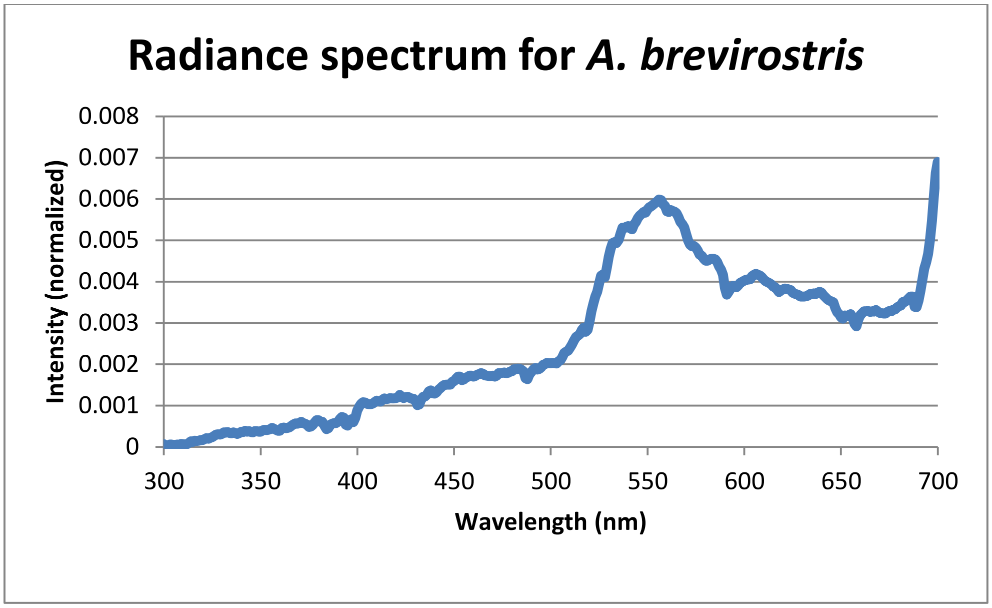


Figure 4: The average radiance spectrum of the light habitat for *A. brevirostris*.

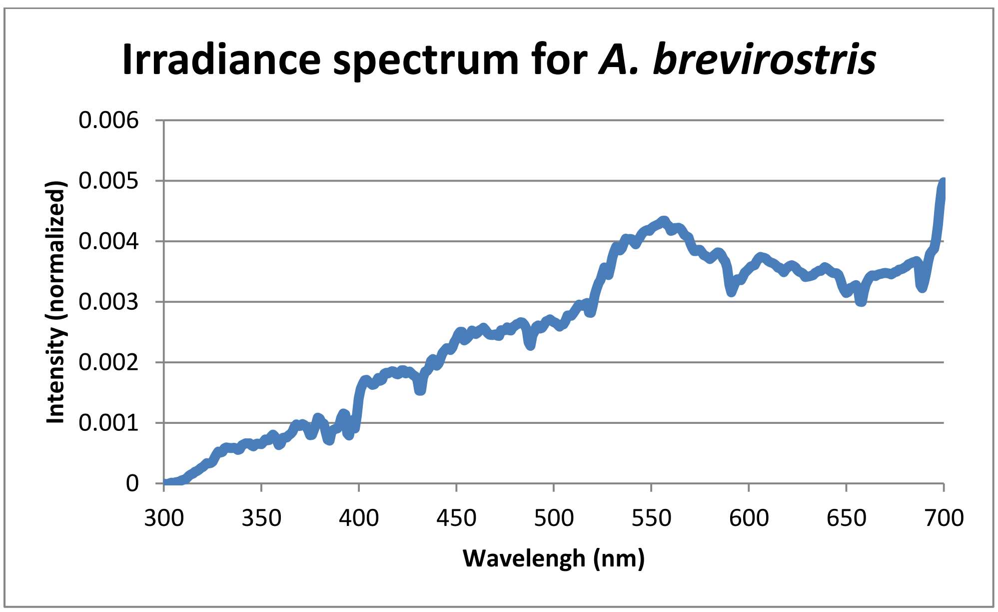


Figure 5: The average irradiance spectrum of the light habitat for *A. brevirostris*.

Overall 20 specimens were collected for this species and the dewlap color, body color and background can be seen plotted together in tetrahedral perceptual color space in Figure 6. The dewlap color and the background do not overlap, while the body color and the background do.

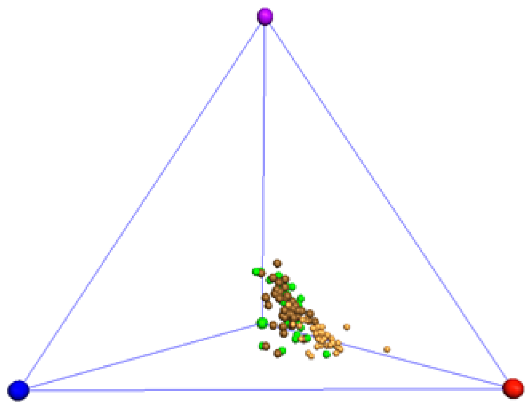

Figure 6: Dewlap color, body color and background for *A. brevirostris* plotted together in tetrahedral perceptual color space. The light orange dots represent the dewlap color, the brown dots represent the body color, and the green dots represent the background.

***Anolis coelestinus***

A picture of *A. coelestinus* can be seen in Figure 7.


Figure 7: An *Anolis coelestinus* specimen with dewlap extended.

There were 45 light habitats measured overall for this species and there is a large peak in both spectra between 530-570 nm. The average spectra of the radiance and irradiance of their light habitat can be seen in Figures 8 and 9.

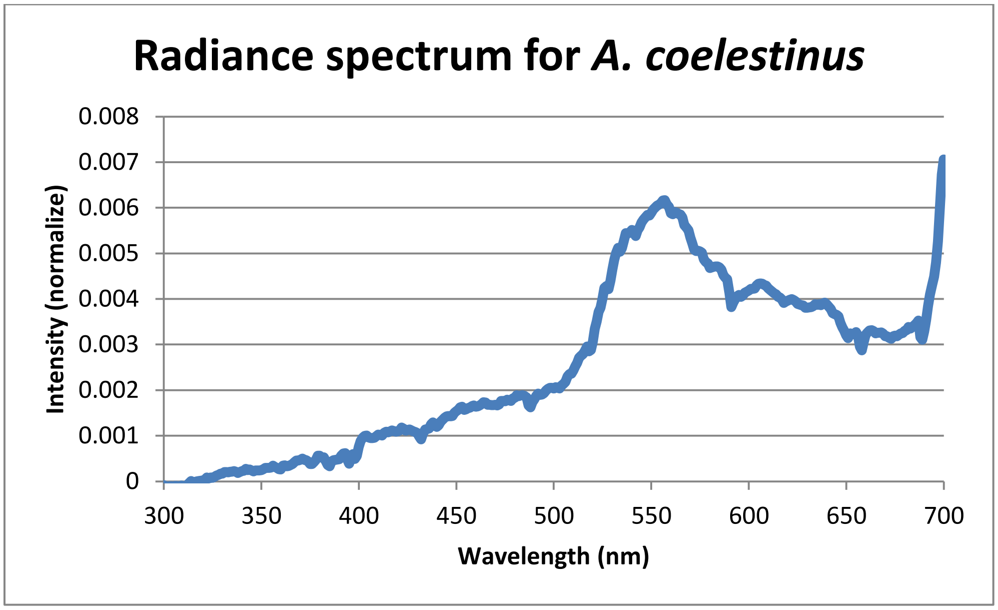


Figure 8: The average radiance spectrum of the light habitat for *A. coelestinus*.

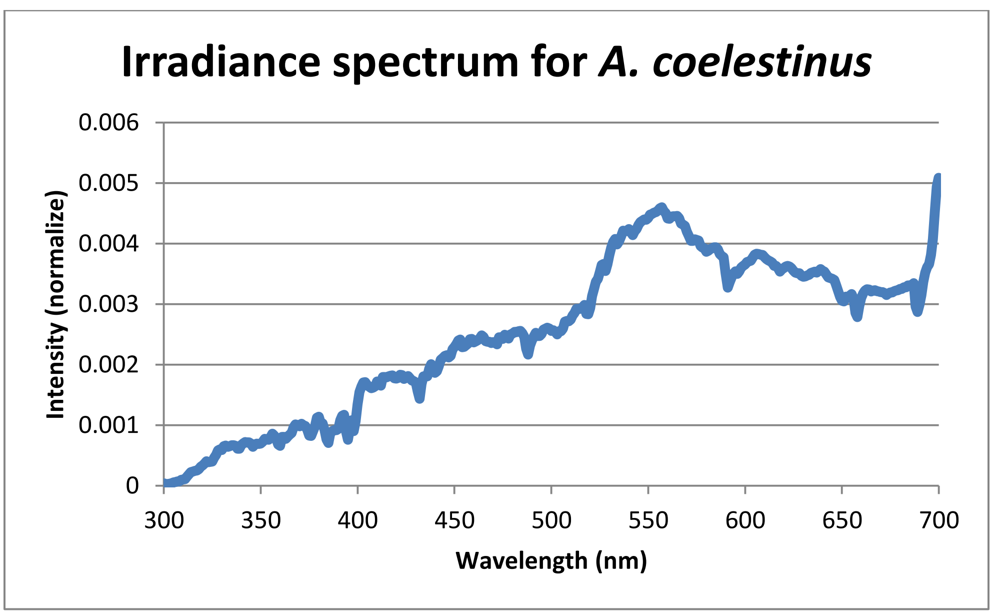


Figure 9: The average irradiance spectrum of the light habitat for *A. coelestinus.*

Overall 20 specimens were collected for this species and the dewlap color, body color and background can be seen plotted together in tetrahedral perceptual color space in Figure 10. The dewlap color and the background do not overlap, while the body color and the background do.

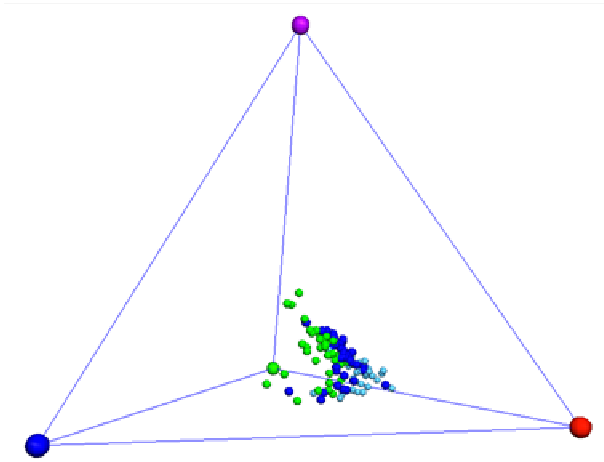

Figure 10: Dewlap color, body color and background for *A. coelestinus* plotted together in tetrahedral perceptual color space. The light blue dots represent the dewlap color, the blue dots represent the body color, and the green dots represent the background.

### *Anolis cybotes*

A picture of *A. cybotes* can be seen in Figure 11.

Figure 11: An *Anolis cybotes specimen* with dewlap extended.

There were 37 light habitats measured overall for this species and there is a large peak in both spectra between 530-570 nm. The average spectra of the radiance and irradiance of their light habitat can be seen in Figures 12 and 13.

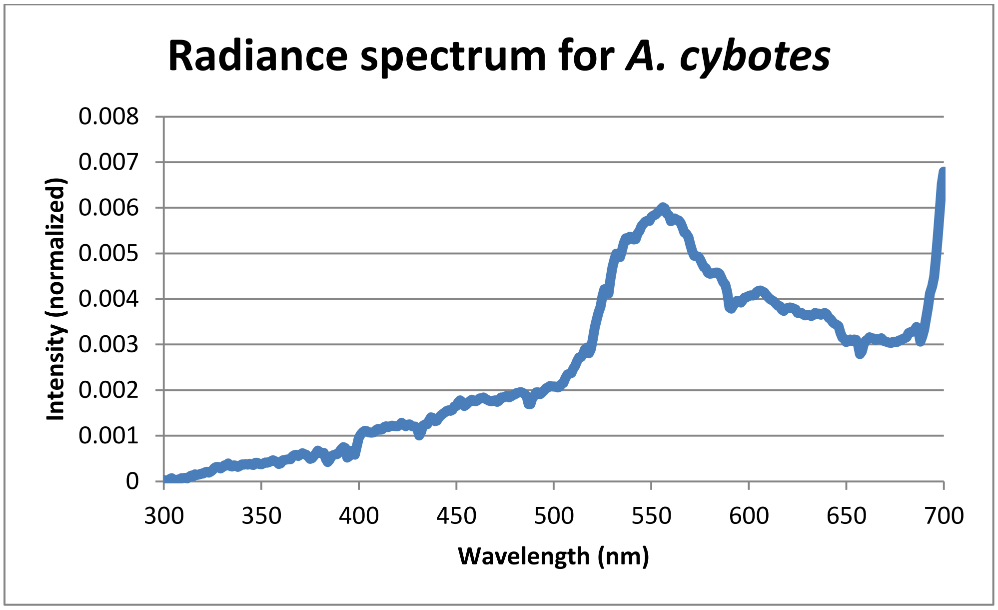


Figure 12: The average radiance spectrum of the light habitat for *A. cybotes*.

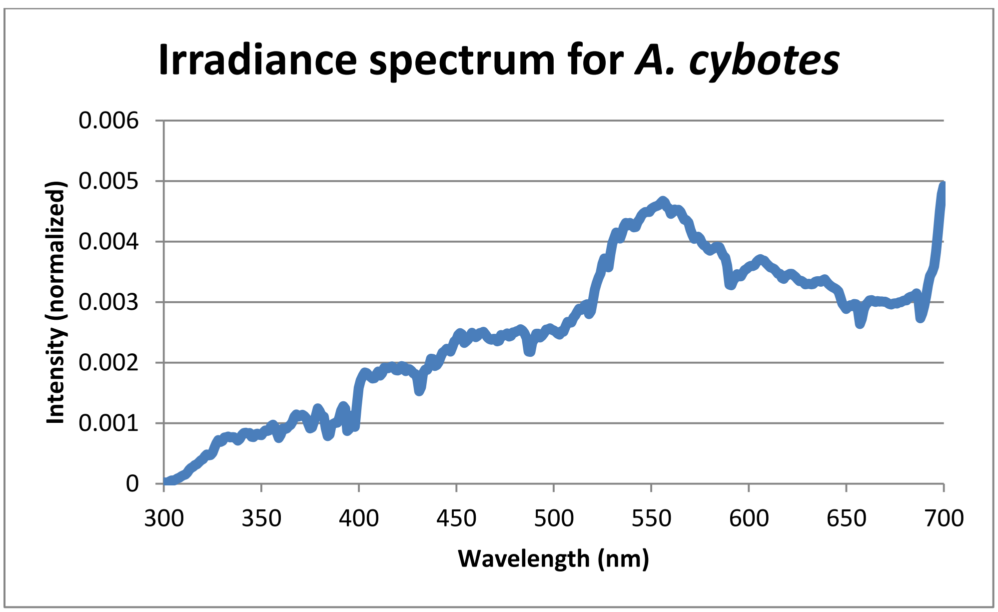


Figure 13: The average irradiance spectrum of the light habitat for *A. cybotes*.

Overall 20 specimens were collected for this species and the dewlap color, body color and background can be seen plotted together in tetrahedral perceptual color space in Figure 14. The dewlap color and the background do not overlap, while the body color and the background do.

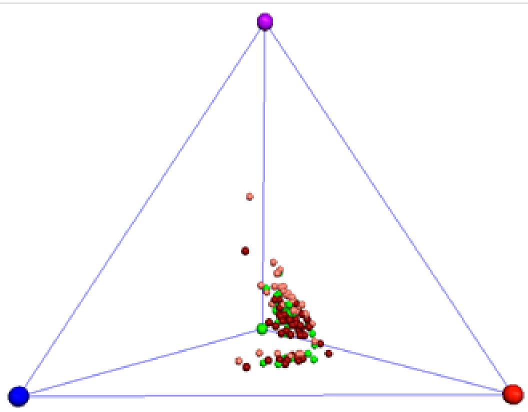

Figure 14: Dewlap color, body color and background for *A. cybotes* plotted together in tetrahedral perceptual color space. The pink dots represent the dewlap color, the dark red dots represent the body color, and the green dots represent the background.

***Anolis distichus***

A picture of *A. distichus* can be seen in Figure 15.

Figure 15: An *Anolis distichus* specimen with dewlap extended.

There were 19 light habitats measured overall for this species and there is a large peak in both spectra between 530-570 nm. The average spectra of the radiance and irradiance of their light habitat can be seen in Figures 16 and 17.

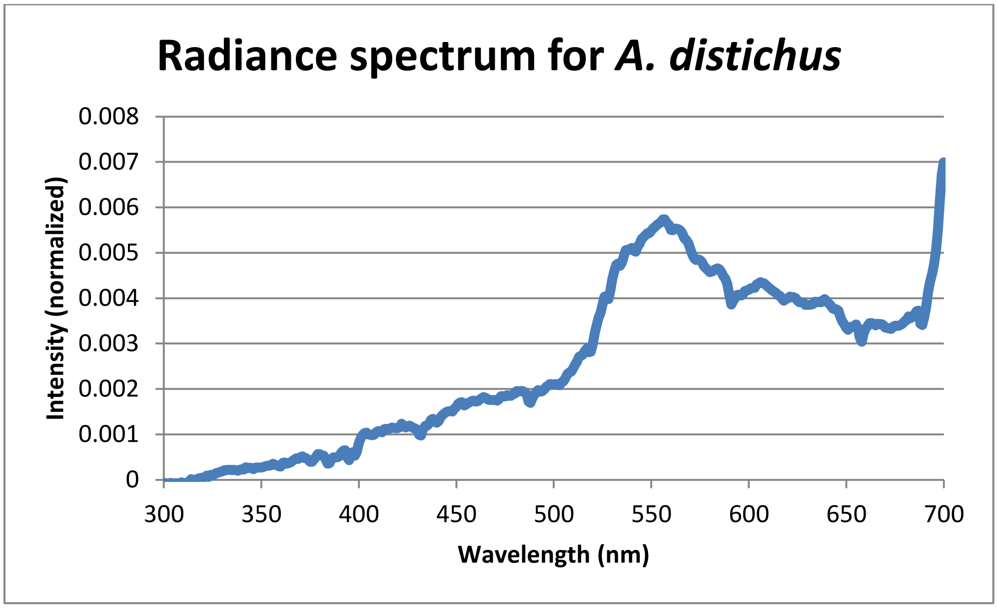


Figure 16: The average radiance spectrum of the light habitat for *A. distichus*.

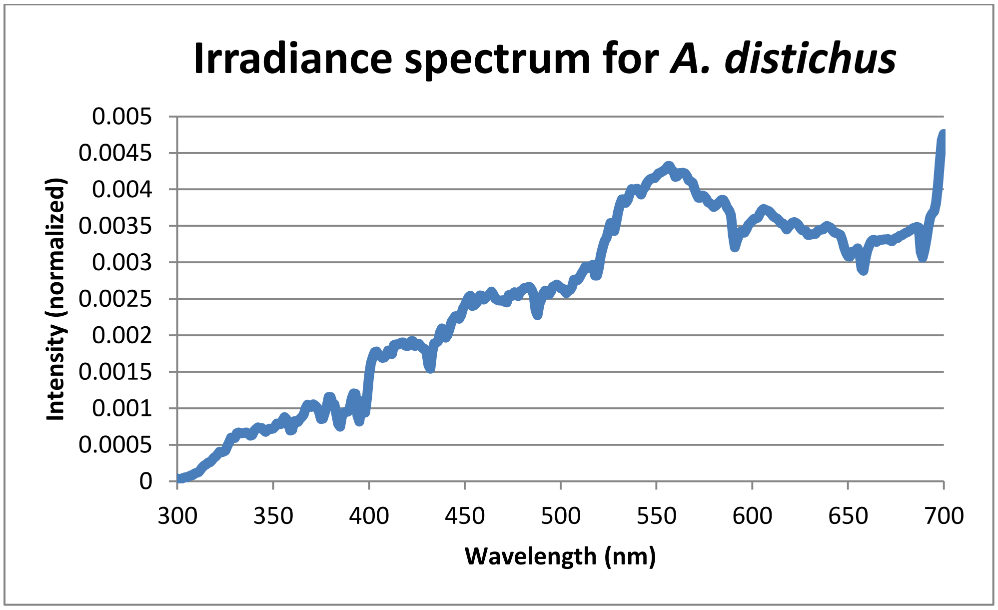


Figure 17: The average irradiance spectrum of the light habitat for *A. distichus*.

Overall 20 specimens were collected for this species and the dewlap color, body color and background can be seen plotted together in tetrahedral perceptual color space in Figure 18. The dewlap color and the background do not overlap, while the body color and the background do.

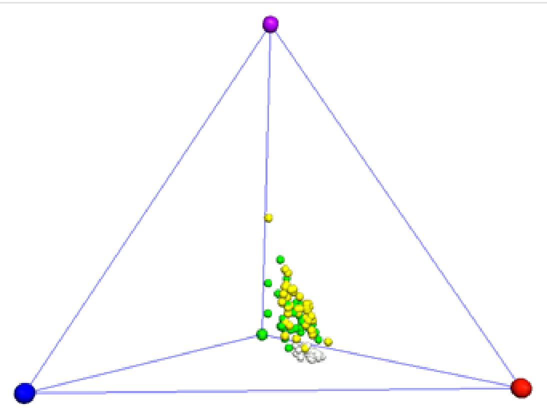

Figure 18: Dewlap color, body color and background for *A. distichus* plotted together in tetrahedral perceptual color space. The white dots represent the dewlap color, the yellow dots represent the body color, and the green dots represent the background.

***Anolis olssoni***

A picture of *A. olssoni* can be seen in Figure 19.

Figure 19: An *Anolis olssoni* specimen with dewlap extended.

Relatively few were spotted, most likely due to a drought, so as a result sample areas were measured where few had been seen before. There were 14 sample light habitats measured overall for this species and there is a large peak in the radiance spectrum between 530-570 nm, but in the irradiance spectrum the spectrum begins to flatten out around that wavelength. The average spectra of the radiance and irradiance of their light habitat can be seen in Figures 20 and 21.

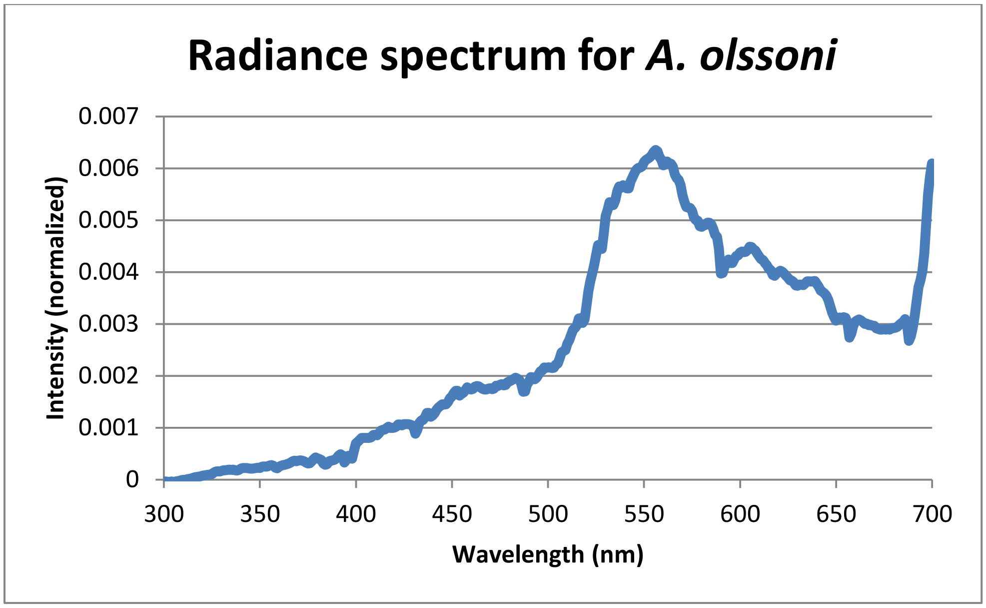


Figure 20: The average radiance spectrum of the light habitat for *A. olssoni*.

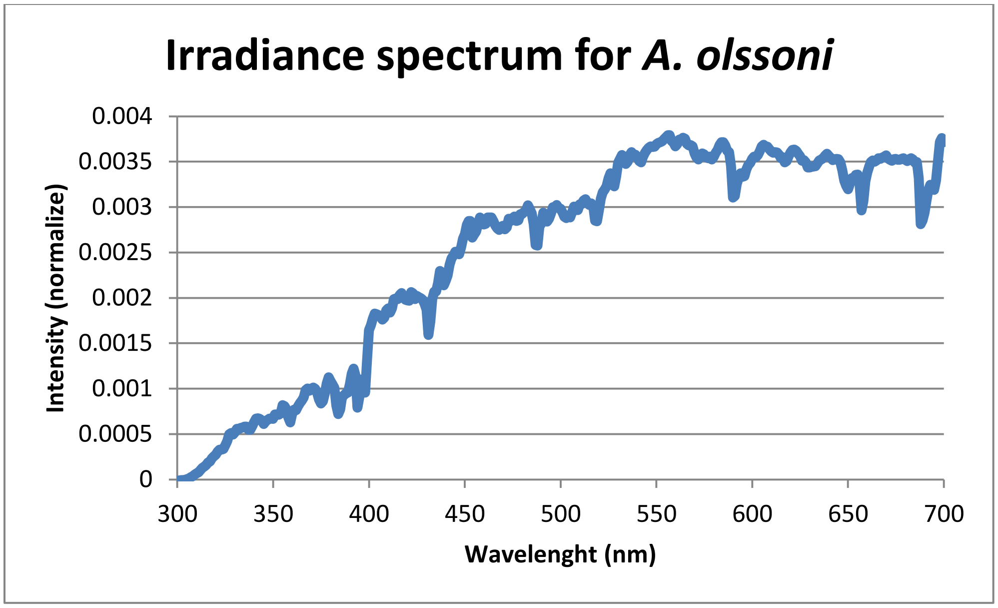


Figure 21: The average irradiance spectrum of the light habitat for *A. olssoni*.

There is a large peak in the radiance spectra between 530-570 nm, but in the irradiance spectra the spectra begins to flatten off. Only 2 specimens were collected for this species and the dewlap color, body color and background can be seen plotted together in tetrahedral perceptual color space in Figure 22. There is no respective overlap between the background, the dewlap color or the body color.

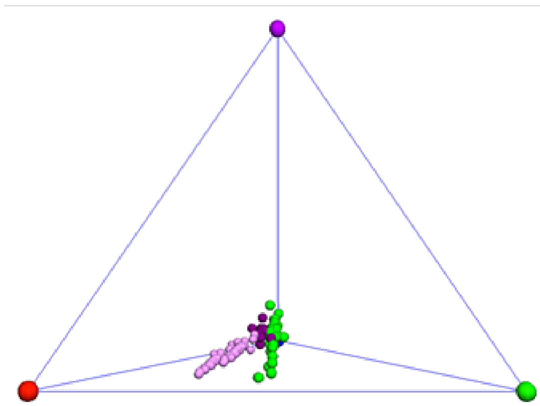

Figure 22: Dewlap color, body color and background for *A. olssoni* plotted together in tetrahedral perceptual color space. The light purple dots represent the dewlap color, the purple dots represent the body color, and the green dots represent the background.

## Comparison of light habitats between different species

### Radiance

A graph with the overlapping normalized radiance spectra of the five species can be seen in Figure 23. They all show the same general overlap and a peak located between 530-570 nm.

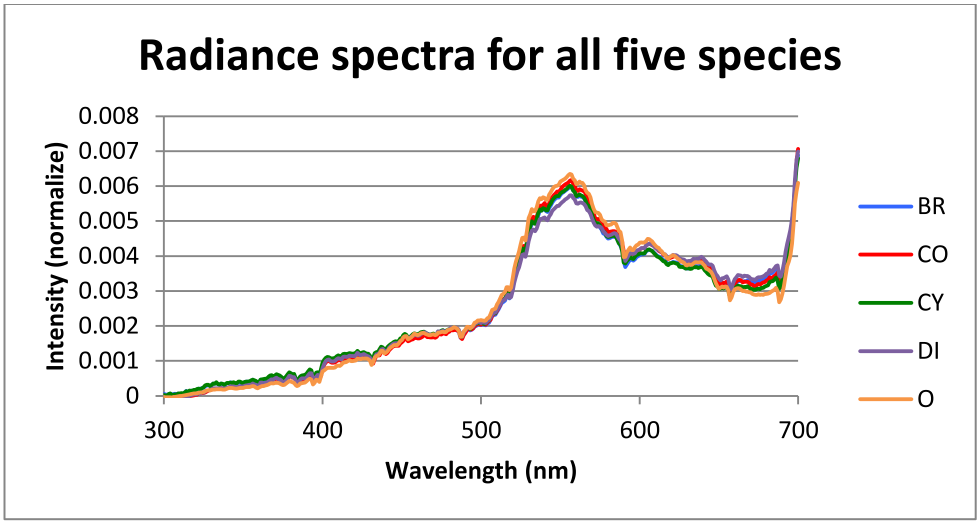


Figure 23: The average radiance spectra of the light habitat for all five species. BR represents *A. brevirostris*, CO represents *A. coelestinus*, CY represents *A. cybotes*, DI represents *A. distichus*, and O represents *A. olssoni*.

**Irradiance**

A graph with the overlapping irradiance spectra of the five species can be seen in Figure 24. They all show the same general overlap and a peak located between 530-570 nm except for *A. olssoni*. This species shows a much broader spectrum and doesn't show a peak between 530-570 nm, but rather flattens out.

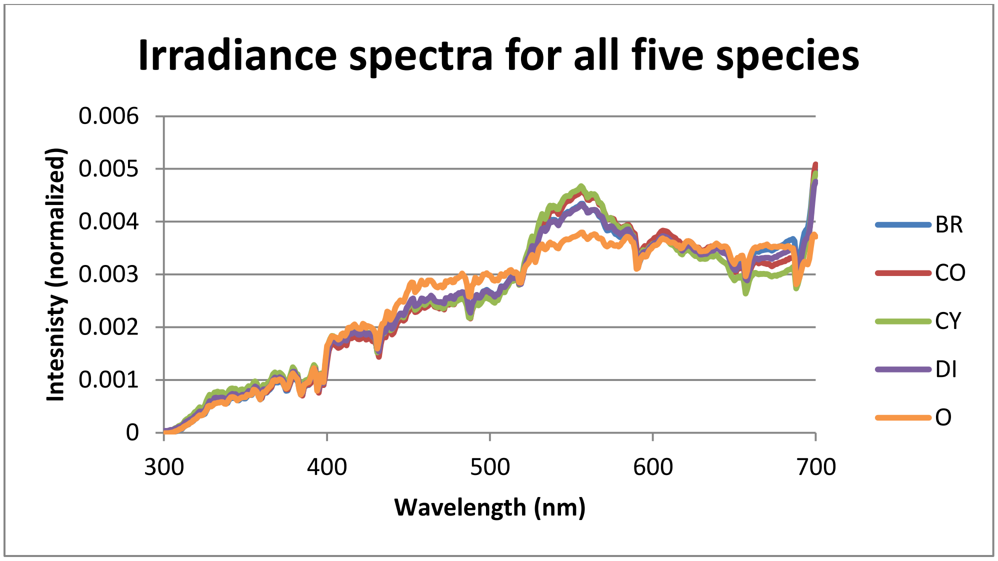


Figure 24: The average radiance spectra of the light habitat for all five species. BR represents *A. brevirostris*, CO represents *A. coelestinus,* CY represents *A. cybotes*, DI represents *A. distichus*, and O represents *A. olssoni*.

**Comparison of dewlap colors between species**

The data of the dewlap colors of the five different species can be seen graphically in a tetrahedron in Figure 25. There seems to minimal overlap between the different data points between the different species.

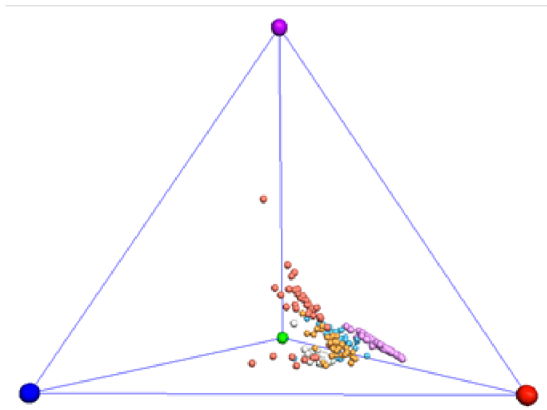

Figure 25: The dewlap color for all five species plotted together in tetrahedral perceptual color space. The light orange dots represent *A. brevirostris*, the blue dots represent *A. coelestinus*, the pink dots represent *A. cybotes*, and the white dots represent *A. distichus*, and *A. olssoni*.

**Discussion:** Through the use of our feeding assay we were able to show that color contrast was more important for *A. sagrei* than luminance contrast in distinguishing food from the background. Our results for experiment 1 were consistent with our hypothesis since the majority of the lizards chose the worm that didn't match the background and this supports the idea that color contrast is very important for anoles. With the addition of luminance contrast in experiment 2, our results were consistent with our hypothesis although there was a slightly greater probability of the lizard choosing the worm that matched the background. The reason for this slight difference is most likely due to a color preference being created and this has been shown to occur in chickens as well in experiments (Hanm and Osorio 2007). However, after more trials the bias would disappear. Finally with regards to experiment 3, although we were unable to obtain results, we know what aspects to improve upon to get results in the future. Some possible aspects that could be improved could be to train the lizards to eat colored worms in a variety of light conditions so that they are not so adjusted to eating in one set light condition. In addition,

we could provide slightly better light conditions in the experiment because it seemed that the lizards were unable to even see the worms, let alone discriminate between the two different colored worms in the low light conditions. So the overall experiment supports the idea that color contrast is very important for signal visibility for anoles in the wild.

In addition, the overall experiment showed that the methods we used produced results effectively for anoles. It also required very little resources and time as well. Therefore, this is an effective approach to testing signal visibility in an animal that is difficult to study with standard conditioning techniques like anoles. This could serve as a possible model as well for testing signal visibility in other animals that are difficult to study with standard conditioning techniques.

With regards to the field data, we determined that the light environment was very similar in spectra across the five different *Anolis* species. They all had similar irradiance and radiance spectra all with a relative peak between 530-570 nm and this peak makes sense since there was a great amount of green vegetation in the areas where we measured their light habitats. The only species that varied at all with regards to the background was *A. olssoni* whose radiance spectrum flattened out around 530nm. The most likely reason for this is due to this species being a grass anole and living in more unshaded areas than the others. It is also important to mention that their radiance spectrum is very similar to the spectrum of sunlight.

Then when taking into account the dewlap colors of each species and their backgrounds, they didn’t seem to show any overlap in perceptual color space. However, the body colors were more similar to the background than the dewlap colors and there was also very little overlap between the body and dewlap color in each species. This shows the importance of body color as camouflage and the dewlap being quite different than the background for signal visibility. In addition, the dewlap colors between each species didn’t overlap at all. Although it has been

shown that in order to maximize signal visibility there needs to be significant variation between the background and visual signal, this suggests that much more is going with regards to the evolution of dewlaps in anoles (Fleishman 2000). As stated before, the anoles all had similar backgrounds and this was most likely the initial driving force for color variation in dewlap color in anoles in the Dominican Republic. However, it is most likely that there is such variation in dewlap color between the five different *Anolis* species so that they can distinguish each other from other species of anoles and this has been suggested in other studies as well (Fleishman 2000). But it is important to mention that there are some restraints on the evolution of dewlap color such as the overlap of the MWS and LWS cones that prevents one of these cones being more stimulated than the other. Finally another restraint in dewlap color could be the mechanisms of skin pigmentation and the colors they produce and it has been shown that there are different mechanisms that produce different colored skin pigments in Jamaican anoles (Macedonia et al. 2000).

Overall, the experiments and field data both support the idea that contrast with the background is very important for anoles for signal visibility. Maximizing signal visibility with the background has been an important evolutionary factor so that they are able to perform their fundamental life processes. Although this study focused primarily on *Anolis* species from the Dominican Republic, this same observation could probably be made for *Anolis* species on other Caribbean islands and has been made on islands such as Puerto Rico and Jamaica (Losos 2009, Fleishman et al. 2009). There are many other species of animals where signal visibility is very important and this data on anoles provides insight for how other species of animals may have evolved.

## References

Bradbury, J.W. and Vehrencamp, S.L. (1998). “Principles of Animal Communication.” *Sinauer Associates, Inc.* Print.

Fleishman, L. J., Loew, E. R., and Leal, M. (1993). “Ultraviolet vision in lizards.” *Nature* 365:397. Web.

Fleishman, L. J. (2000). “Signal function, signal efficiency and the evolution of anoline lizard dewlap color” pp. 209-236. In: Y. Espmark, T. Amundsen, and G. Rosenqvist (eds.), Animal Signals: Signalling and Signal Design in Animal Communication. Tapir Academic Press, Norway. Print.

Fleishman L.J., Persons, M. (2001). “The influence of stimulus and background colour on signal visibility in the lizard *Anolis cristatellus*.” *Journal of Experimental Biology* 204:1559-1575. Print.

Fleishman, L. J., Leal, M. and M. Persons (2009) “Habitat light and dewlap color diversity in four species of Puerto Rican anoline lizards.” *Journal of Comparative Physiology* A 195: 1043-1060

Fleishman, L. J., Ogas, B., Steinberg, D. and Leal, M (2015). “Why do *Anolis* dewlaps glow? An analysis of a translucent visual signal.” *Functional Ecology*, 30: 345–355. Web.

Ham, A.D., and Osorio, D. (2007). “Colour Preferences and Colour Vision in Poultry Chicks.” *Proceedings of the Royal Society B: Biological Sciences* 274.1621: 1941–1948. *PMC*. Web.

Kemp, Darrell J., Herberstein Marie E., Fleishman Leo J., Endler John A., Bennett Andrew T. D., Dyer Adrian G., Hart Nathan S., Marshall Justin, Whiting Martin J., Associate

Editor: Edmund D. Brodie III, and Editor: Susan Kalisz (2015). "An Integrative Framework for the Appraisal of Coloration in Nature." *The American Naturalist* 185.6: 705-24. Print.

Losos, J.B. (2009). “Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles.” Berkeley: University of California Press. Print.

Macedonia, J.M., James, S., Wittle, L.W., and Clark, D.L.(2000) Skin Pigments and coloration in the Jamaican Radiation of Anolis Lizards. Journal of Herpetology 34: 99-109. Web.