Temperature and Direction-changing Effects on the Agonistic Headbob Displays of *Anolis sagrei*

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BIO Honors Senior Thesis

Introduction

For a communication signal to be effective, it must be able to grab the attention of any onlooker while also conveying information that can affect the behavior of the onlooker (Fleishman 2019). If a signal response is, on average, beneficial to both the signaler and receiver, then a stable signal and signal system will evolve (Fleishman 2019). The physical properties of the signal are constrained by the sensory system, which in many animal groups has been conserved throughout evolutionary history (Fleishman 1992; 2019). The process in which a sensory system influences the structure (the physical nature) of a signal is called sensory drive (Endler 1992; Fleishman 1992). Sensory drive primarily concerns how a sensory system affects the physical structure rather than the design, or purpose, of a signal (Endler 1992). The physical nature of the signal needs to be able to exploit the sensory system of the receiver in order to gain attention, which potentially results in a variety of different behaviors (mating, aggression, food acquisition) depending on the information the signal conveys (Endler 1992; Fleishman 1992). Thus, sensory systems, signals, and behavior all relate to each other and influence each other evolutionarily (Endler 1992). A change in one aspect will likely change the other aspects of communication signaling (Endler 1992).

Signals are also directly impacted by their environment (e.g., shady vs. sunny habitats) (Fleishman 2019). The physical environment introduces two intrinsic problems to signal communication: the inattentiveness of a receiver, and the varying distance from the signaler to the receiver (Steinberg 2013). In order for the signal to be effective, it has to be able to grab the attention from a receiver while also accounting for changes in distance. Recall that the signal also has to have a net benefit to both the signaler and receiver, on top of needing to be detectable in the environment and the sensory system in which the signal exploits. The evolutionary

mechanisms involved in establishing a stereotyped signal are constrained by the physical nature of the environment and the sensory system of any species.

A signal must carry information about the signaler or its intentions in order to be effective. The intention communicated by a signal must reflect the cost of producing that signal. This is called the 'handicap principle,' which states that the honesty in a signal is maintained by the cost of producing that signal (there must be a net-benefit to the higher-quality individual) (Higham 2013; Polnaszek 2014). A signal is honest when it correlates with the physical properties or intentions of the receiver. If the costs are low for the opportunity to fake a signal or cheat, then the signalers will likely signal dishonestly, and vice versa (Polnaszek 2014). However, in such cases the receiver is less likely to respond to that signal because there is less evolutionary pressure to respond. There are many potential aspects and costs that maintain honesty in a signal. Dishonest signaling may result in retaliation towards the signaler, especially in agonistic or territorial disputes (Higham 2013). If a smaller individual fakes a signal to try and scare off a larger individual, the larger individual may assess this as dishonest and retaliate. However, the signal does not have to be dishonest for the receiver to retaliate. The cost of dishonest signaling is that the dishonest signaler is not prepared to sustain the cost of retaliation.

Another potential mechanism that maintains honesty is that some signals cannot physically be faked, such as size-related vocal signals in certain amphibians (Higham 2013). Another mechanism that can lead to the evolution of honest signaling is when there is an alignment of interest between the signaler and the receiver (Higham 2013). If there is mutualistic interest between the signaler and the receiver, there may exist no cost to signaling (Higham 2013). However, this particular study only deals with agonistic signaling, so mutualism will not

be discussed any further. This study is specifically about the agnostic honest signaling of *Anolis sagrei* headbob displays.

The genus *Anolis* contains 416 different species with a nearctic and neotropical distribution, and is the only member in Dactyloidae (Losos 2011; Rodda 2020). They are part of the infraorder Iguania, which is the sister group to all other Squamates. They are also found throughout the greater and lesser Antilles and have natural distribution as far as Asia, as well as human-mediated distribution (Losos 2011). They eat a wide array of food and lack developed chemoreception, a common trait of all Iguanids (Losos 2011). The lack of a developed chemoreceptive sense is due to differences in prey acquisition between Iguanids and Scleroglossans (Baekens 2016). Iguanids retained lingual prehension, as opposed to jaw prehension that's seen in Scleroglossa (Baekens 2016). This means they never developed the forked tongues that are typically seen in reptiles with developed chemoreception (not all Scleroglossans have forked tongues, however) (Baekmans 2016).

Almost all male anoles possess a dewlap, which is a colorful retractable structure on the throat controlled by the hyoid apparatus (Losos 2011). The dewlap is used for communication in a variety of contexts (i.e., aggression and courtship) as well as a way to differentiate between species (Losos 2011). Anoles are visually-oriented animals, which explains their lack of chemoreception for predation as they only eat moving prey (Fleishman 1992; Losos 2011). Anoline vision is based on temporal patterns rather than structural patterns, meaning that their response isn't restricted by the shape of an object, and they can respond to anything that is able to grab their attention through motion (Fleishman 1992). The anoline eye has a 200° extent, with 180° monocular vision laterally and 20° binocular overlap in front (Fleishman 1992). Anoles lack rods, meaning they have high acuity in high-intensity light since they are diurnal (Fleishman

1992). The anoline photoreceptors populate the retina, which is divided into three different regions: the peripheral retina, the central fovea, and the temporal fovea (Fleishman 1992). The central fovea has the highest density of photoreceptors with a minimum resolvable angle of 0.008° (Fleishman et. al 2017) . The retina is especially important in signal reception because it, along with the optic tectum, are involved with sensing motion signals after the signals trigger the visual grasp reflex (Fleishman 1992; Fleishman 2019). The optic tectum integrates information from eye neurons and connects it to motor neurons to elicit a change in gaze and attention (Fleishman 2019). Motion is detected with the peripheral retina and the eye shifts to the central fovea to clearly see the image (Fleishman 1992). However, not all motion causes a shift in attention for anoles (Fleishman 1986; Fleishman 1992; Steinberg 2013).

Anoles are sensitive to certain types of motion based on frequency, timing, and amplitude (Fleishman 1992; Steinberg 2013). Anoles, on average, won't respond with equal probability to all types of motion. Anoles live in heavily-forested environments, where the background vegetation is almost constantly moving due to wind or other outside forces. If anoles responded to all types of motion equally, then their sensitivity to prey movement would be the same as their sensitivity to vegetation movement. This would make them inefficient predators. So, anoles react to movement of certain frequencies and waveforms in order to distinguish relevant stimuli for survival from irrelevant (Fleishman 1986). Background vegetation moves in a sinusoidal wave pattern of varying frequencies and amplitudes (Fleishman 1986). Fleishman (1986) found that *Anolis auratus* reacted significantly more to stimuli that moved in a square-wave at high velocity and acceleration, which is movement that is unlike the background vegetation. However, this is not consistent in all instances (Fleishman and Pallus 2010). Fleishman and Pallus (2010) found that high acceleration and high velocity were not always more effective at eliciting attention than

lower speeds and acceleration. Interestingly, Fleishman (1986) found that the visual grasp reflex (VGR) of *A. auratus* is dependent on the amplitude of motion, not necessarily the velocity and frequency. Higher amplitude motion, which has a higher velocity and acceleration, was less likely to elicit a response (Fleishman and Pallus 2010). This situation shows why high velocity and acceleration don't always make a response more likely. *A. auratus* is most sensitive to an amplitude of 0.22° or more, but this threshold ranges from 0.25-0.75° for *Anolis gundlachi,* suggesting that different species of anoles react to certain thresholds more sensitively than others (Fleishman 1986; Steinberg 2013). Fleishman and Pallus (2010) constructed a two-dimensional motion detector (2DMD) model to predict changes of attention in *Anolis sagrei* using data from *A. sagrei* and other *Anolis* species. The model indicated that abrupt movements of less than 100 ms and an amplitude of 0.2-0.4° were optimal parameters to elicit a response (Fleishman and Pallus 2010). This is consistent with live animal responses, which shows that the model is fairly accurate in predicting attention responses in *Anolis*.

Anoles are able to react to certain motion stimuli differently in part due to their adaptation and habituation to motion (Fleishman 1986). Adaptation to a certain stimulus is when a species fails to respond with repeated stimuli, while habituation is when a species ceases to respond to certain stimuli, but reacts to novel stimuli (Fleishman 1986). Anoles likely habituate to background vegetation movement since adaptation to any stimulus reduces the responsiveness of any second motion pattern, even if it's different (Fleishman 1986). The anoles would cease to respond to any motion stimuli if they did not adapt to the background motion. Instead, they habituate to sinusoidal motion patterns to reduce their sensitivity to that form of movement. The VGR relies on two processes to avoid irrelevant motion: preference for certain motion patterns, and short-term habituation to specific motion patterns (Flieshman 1986). Since motion-sensitive

cells in the eyes are velocity-tuned, they habituate to rapid movement (Fleishman 2019). However, since anoles habituate and not adapt to stimuli, the motion-sensitive cells develop sensitivity again 20-30 seconds after the rapid stimulus (Fleishman 1986; Fleishman 2019). Anoles don't only use motion for finding prey; they also use it for communication, so a sensitive and accurate motion detection sensory system is imperative for survival.

A very important signal that *Anolis* lizards use to communicate both mutualistic and agonistic intentions are their headbob displays. Jenssen (1977) defines a headbob display as a highly stereotyped movement of the anoline head and/or dewlap, typically accompanied by movement of the legs and tail in conjunction with the head. The display must be stereotyped and shared by the population (Jenssen 1977). Jenssen (1977) reviews the possible ways in which the headbob displays evolved, and suggests that it did not evolve *de novo*. Jenssen (1977) discusses three different possibilities for the evolution of the headbob displays. The first is the evolution from non-communicative locomotion (Jenssen 1977). The neural pathways would already be established for the displays to evolve (Jenssen 1977). Another possibility is that it evolved from a thwarting behavior (Jenssen 1977). Thwarting is a response of the somatic nervous system to displace energy into different behaviors or movements when there is an obstruction to arousal (Jenssen 1977). It's possible that movement of the head was originally a thwarting behavior that evolved to become the headbob displays. For the last possible origin that Jenssen (1977) talks about, they use the agonistic interactions between blue spiny lizards (*Sceloporus cyanogenys*) as an example. When a dominant male spiny blue lizard raises its head, the subordinate will lower its head in submission (Greenberg 1977). This interaction, as well as the anoline headbobbing, could have evolved from a common agonistic behavior of basal lizards (Jenssen 1977; Greenberg 1977). Anoles also perform a series of rapid, weakly-stereotyped headbobs called "jiggling"

(Jenssen 1977). Jiggling is different from the actual headbob displays since it is not typically stereotyped, it has no distinct beginning or end, and only seems to communicate arousal (Jenssen 1977). It is a primitive behavior that could potentially be a clue to how the headbob displays evolved. The origin is not entirely known and more phylogenetic studies need to be conducted to better understand the origins of these displays.

When presenting to other anoles, the presenters orient themselves laterally so they can maximize visual acuity and gain the attention of the conspecific (Jenssen 1977). The anoline body has evolved ways to exaggerate lateral structures during displays in order to better communicate, such as having a laterally-compressed body, erecting the nuchal crest, and elevating the body with an arched back (Jenssen 1977). Since motion patterns in visual displays draw attention and transmit information, orienting one's body to better sense the signal makes communication much more efficient (Fleishman 1992). Recall that to draw attention, it has to stimulate the peripheral retina, which will trigger the VGR if the lizard is sensitive to that type of motion (Fleishman 1986; Fleishman 1992). The displays are used to communicate in a variety of different situations such as species identification, mate attraction, and agonistic interaction (Fleishman 1986). Display movements typically occur on the y-axis since that is how the muscles and neurons are oriented (Jenssen 1977). Since a requirement for these displays is stereotypy, each species of anole has its own unique display-action-pattern (DAP) that is consistent within that species (Jenssen 1977). Recall that displays are used for species recognition, so stereotypy would be evolutionarily favorable for females to recognize conspecifics (Jenssen 1977). The stereotyped portion of the display is called the core, and it appears in every single display of its respective species, and holds flexible meaning (Jenssen 1977). The core is predicted to not occur in the beginning of the display since that is the least

stereotyped portion of the display (Fleishman 1992). This is because it is likely for an inattentive viewer not to see the beginning portion of a display, so there is low selective pressure for stereotypy in the beginning (Fleishman 1992). The core variability doesn't transmit enough information alone, so lizards add modifiers to their displays to signal their exact intentions (Jenssen 1977). These modifiers are typically different postures or motion patterns (tail-flicking, dewlap extension, temporal changes with head movement, etc.) that are shared by a population (Jenssen 1977). Modifiers can be either dynamic or static based on what the lizard changes (Jenssen 1977). Static modifiers are actions that don't involve varying head movements (sticking out the tongue, dewlap extension, etc.), while dynamic modifiers are differing movement patterns of the head and body (Jenssen 1977). Interestingly, modifiers are shared interspecifically since many are primitive and shared throughout the family *Iguanidae*, which means different species can use these modifiers to communicate (Jenssen 1977). Variability in anole DAPs is useful for species recognition and signal intensity (Jenssen 1977). If most of the variability exists between lizards instead of within an individual, then intra-individual stereotypy exists (Jenssen 1977). Jenssen (1977) concludes that large core variability shows intra-individual stereotypy, which is important for individual recognition. Jenssen (1977) shows how all male *Anolis nebulosus* have identically patterned signature displays, but they temporally differ in their core display. So, temporal variability in the core display functions as individual recognition clues based on duration (Jenssen 1977). It makes sense that temporal variability acts as individual recognition clues since changes in frequency or intensity change with distance, and thus aren't stereotyped for individuals (Fleishman 2019). Modifier variability indicates intra-population variability, since any lizard in a given species can perform a common repertoire of modifiers

(Jenssen 1977). Variability in these modifiers thus creates a gradient of display intensity that is recognizable between species and populations (Jenssen 1977).

Anoles can typically have more than one display pattern in their repertoire that differs based on social context (Jenssen 1977). Two examples are the challenge displays and the assertion displays (Fleishman 1992). Both displays are present in anoline display repertoires, but are used in different contexts and have different movement patterns and postures (Fleishman 1992). The challenge displays involve the lizard approaching an intruder, orienting themselves laterally, and displaying repetitively with postural modifiers; the assertion display occurs spontaneously in a territory while the lizard is elevated, and the lizard typically adds no postural modifiers or repetitions of the signature display (Fleishman 1992). Jenssen (1977) categorizes the displays using Type A, Type B, etc., with Type A being the signature display and any other pattern being a modified version of the type before it. Type A is a general display, but any other display patterns differ based on the intensity and emphasis of a particular individual (Jenssen 1977). Type B being restricted to agonistic interactions would result in the evolution of honest signaling for that display pattern, since cheating could result in retaliation (Jenssen 1977).

In terms of agonistic behavior, anoles display in their territories both alone and when intruders are present. When an intruder is present, they signal to intimidate the intruder to protect their territory. Recall that honest signals remain honest when they can't be faked or when there are high costs to cheating. Anoles cannot fake their body size, their dewlap size or color, or the functionality of their muscles. They are physically constrained to keep their signals honest. They are also constrained by external costs, such as predation. Displaying with movements that are too abrupt and noticeable could attract a predator, so anoles may not necessarily display using their full abilities (Fleishman 2019). Predation, not energy conservation, is the highest external cost to

displaying (Fleishman 2019). Steinberg et al. (2014) studied how the presence of predators affected displays in *Anolis sagrei*. They found that anoles in territories with more predators significantly reduce their display amplitude and the active space (the distance that a signal can be detected) of the display (Fleishman 1992; Steinberg et al. 2014). The amount of distance present between a signaler and an intended receiver is important information for a signaler to encode in order to gain the attention from the receiver. Agonistic signalers must cope in a variety of different ways in order to effectively signal based on distance (Steinberg & Leal 2013). Signalers either use different motion signals based on distance, or they can modulate the physical properties of a signal (Steinberg & Leal 2013). Anoles at closer distances may elect to use dewlap extension or displays with small amplitudes since it is more likely that the receiver notices a signaler up close than farther away (Fleishman 1992). Unnecessarily displaying with amplitudes that are too high could lead to a greater chance of predation, so the cost of being preyed upon causes signal modulation in anoles (Fleishman 1992). Steinberg and Leal (2013) studied the effects of distance on signal modulation in *Anolis gunlachi* using their headbob displays. They found that headbobbing was the most effective signal at longer distances, and that maximum amplitude and distance are positively correlated (Steinberg & Leal 2013). Recall that the ideal amplitude for headbob displays is around 0.2-0.4°, so a change in distance would require a change in amplitude to better elicit the VGR. The amplitude modulation of *A. gundlachi* was consistent with the model Fleishman and Pallus (2010) proposed, which means signal modulation in *A. gundlachi* is very similar to signal modulation in *A. sagrei* (Steinberg & Leal 2013). Whether this relationship is conserved in all species in the *Anolis* genus is yet to be determined. Steinberg and Leal (2013) also saw amplitude modulation when a signaler wanted to gain the attention of an inattentive lizard. Modulations in amplitude would also cause changes in

velocity and acceleration, however, it is ambiguous whether distance-based modulations in speed are a result of changes in amplitude (Steinberg & Leal 2013). The headbob displays are still restricted based on cost and physical constraints of the muscles, so any modulation is based on these physical constraints.

Temperature is a very important factor in the function of muscles and metabolism in general. Anoles, like all lizards, are ectotherms, which means their body temperature aligns with changes in environmental temperatures. Most *Anolis* species behaviorally thermoregulate, which means they move in and out of light and heat in order to stay at relatively constant temperatures in well-lit and heated environments. Temperature affects both muscle function and associated variables (velocity, acceleration, angle) in both ectotherms and endotherms (Lailvaux 2007). However, endotherms are able to use metabolic heat to maintain a relatively constant body temperature. All animals have an optimal temperature in which their muscles are able to twitch most efficiently, and thus produce work most efficiently. Factors like twitch and tetanic tension and any rate-dependent process (power output, velocity, etc.) are all positively affected by increased temperature to an extent (Bennett 1985). Maximal force output should be temperature-independent since muscles should be able to produce the same amount of force regardless of efficiency (Bennett 1985). Each animal has a thermal tolerance index, which means temperatures that are too high or too low will greatly affect their metabolic and muscular functions (Lailvaux 2007). In Lailvaux's (2007) experiment on how temperature affected jump performance in *Anolis carolinensis*, the thermal sensitivity for all jump performance variables generally correlated, meaning they had similar thermal indices and performed optimally around the same temperature. Interestingly, Lailvaux (2007) found that in the wild, *A. carolinensis* was not at its optimal body temperature, but was a little colder. The ecological reasoning for this

phenomenon is still unsure and requires future research. The combined effects of a suboptimal field temperature and the temperature dependent function of muscles could have some very important implications for anoline headbob displays.

With the ability to analyze headbob displays using cameras that shoot 60 frames per second (fps) comes a more detailed DAP graph of *Anolis sagrei*. Past representations of *A. sagrei* DAPs were very basic in their shape. However, now there is a noticeable difference between past and current DAPs, with the DAP analyzed using a camera with 60 fps having a decrease in amplitude after reaching the peak, followed by a plateau, while the DAP of the camera with less than 60 fps showing a complete square wave shape (Fig 1). After we noticed this difference, we thought about the potential role that this added hump may have in the context of the display. We believed it was either an artifact of the muscle physiology of the lizard that adds nothing to the effectiveness of the display, or it is an evolutionary trait that capitalizes on the *A. sagrei*'s sensitivity of the optic tectum to changes in direction of motion stimuli. The tectal cells are direction-sensitive and are coded to respond strongly to shifts in direction, starts, and stops (Fleishman and Font 2019). Any shift in direction will cause a new group of tectal cells to fire in unison, and this causes a shift in gaze and attention (Fleishman and Font 2019). The bump in the display indicates a quick change in direction at the peak of the headbob display, which may cause any intended receiver to be more likely to notice the signaller's display.

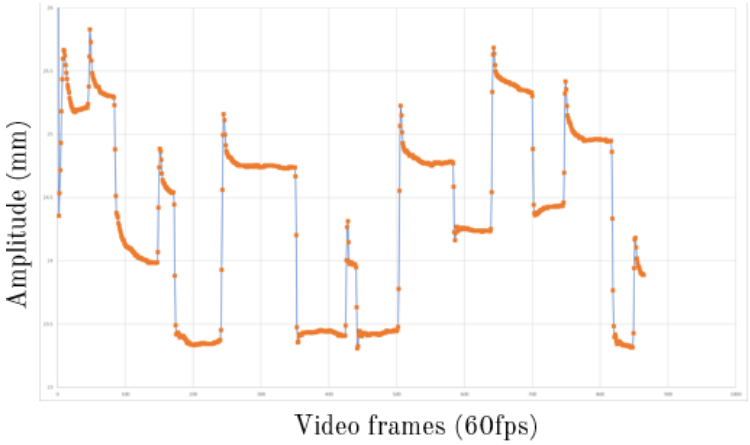

Fig. 1: DAP of *Anolis sagrei* using a Canon Vixia camera, recording at 60 fps. The y-axis shows amplitude (mm) and the x-axis shows time (ms).

In this study, we aimed to see how temperature affected the headbob display in the brown anole, *Anolis sagrei*. We wanted to see if lower temperature significantly affected the temporal pacing of the headbob display. Since muscles cannot fake how warm or cold they are, the headbob displays should be honest signals that communicate to the receiver that the signaller is cold and may not be able to defend their territory as well. We exclusively looked at agnostic territorial displays between an acclimated (in terms of environment) signaler and an introduced receiver. Based on previous research on the effects of temperature on muscle function, we expected there to be a decrease in muscle function efficiency in lower temperatures. However, we also wanted to know if *A. sagrei* was able to detect the decrease in efficiency in the headbob displays. If they can sense a decrease in efficiency, then this could cause a change in aggressive behavior, especially if the signal is honest. We also aimed to look at the purpose of the direction-changing bump in the displays of *A. sagrei*. We hypothesized that this bump was an evolutionary product rather than a physical artifact based on the display patterns of other *Anolis* lizards, which lack the distinctive direction-changing bump (Fig. 2) (Fleishman & Pallus 2010).

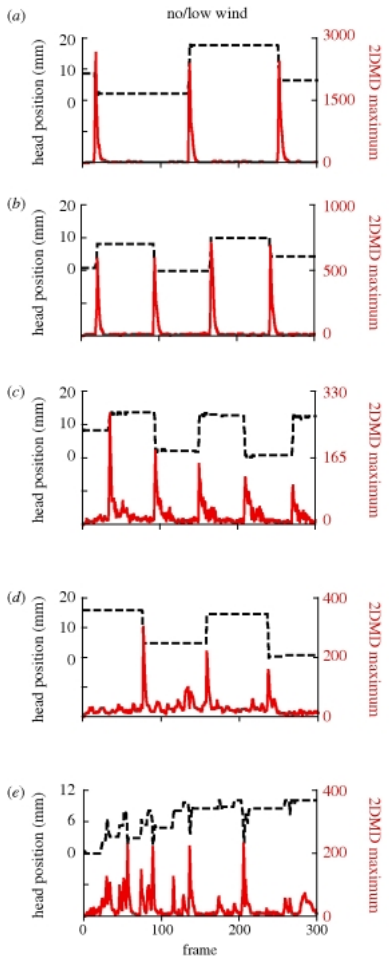

Fig. 2: First 10 seconds of assertion display action patterns of a) *A. gundlachi*; b) *A. cristatellus*; c) *A. pulchellus*; d) *A. krugi*; and e) *A. stratulus* in no/low wind conditions . The black dashed lines show the head position through time. The red line shows the output of a motion-analysis model (Fleishman and Pallus 2010).

Materials and Methods

We conducted two major experiments to look at the effects of temperature on the headbob performance, and to see if *Anolis sagrei* can detect any potential differences in the display motion patterns. We conducted the temperature and rapid movement experiments at Union College in Schenectady, New York from the beginning of September to the end of November. We conducted the direction-changing experiments from February to April at Union College in Schenectady, New York. Each experiment lasted for a total of 10 weeks each. Different lizards

were used for the temperature experiments and the stimulus experiments. The lizards were fed two times per week with crickets covered in Repti Calcium (calcium carbonate and vitamin D3), and watered everyday. Any lizard that was sick or died in the process of the experiment was subsequently replaced, and the experiment carried on.

Temperature Experiment

To see how temperature affected *Anolis* headbob performance, we conducted five different temperature experiments, each with at least six different trials. Three trials were done in "warm conditions," where a Powersun UV 100W self-ballasted mercury vapor UVB lamp and heating lamp were turned on, while three were done in "cool conditions," where the heating lamp was turned off and the light was raised from the cage at least two hours before the trial began. There were two 32x38x41.2 cm cages, each with one lizard, its own Powersun UV 100W lamp, its own heating lamp, a perch laid diagonally across the cage, a solid log on the ground for additional horizontal perching, and rocks on the bottom for the substrate. The cages each had one window on the z-axis and a green background on the -z-axis (Fig. 3). The only opening was on the y-axis on the top of the enclosure (Fig. 3). The two cages were separated with an opaque divider, and the lizards enclosed had no view of each other. Each lizard that remained in the cages habituated to the new environment for at least 1 week. The intruder lizards remained in the same separate room from the display lizards, each in a separate portable clear terrarium. The display lizards on deck also remained in the same conditions as the intruders until they were placed in the display cages. Each terrarium had a perch and substrate, as well as faux foliage to hide (Fig. 3). The temperature in the room was set for 26℃. However we determined near the end of the experiment that the room temperature fluctuated by as much as +/- 3℃.. The amount

of time that each light was on was controlled by a BN-LINK 8 Outlet Surge Protector with Mechanical Timer. The window of the cage faced towards a divider window to the next room, where the Canon Vixia Hf G21 HD camcorder used to record the displays was covered by a blue tarp. The tarp had holes so it covered us recording the displays, but allowed for the camera to see into the room. The cage remained approximately 58.4 cm away from the window that the Canon Vixia filmed through.

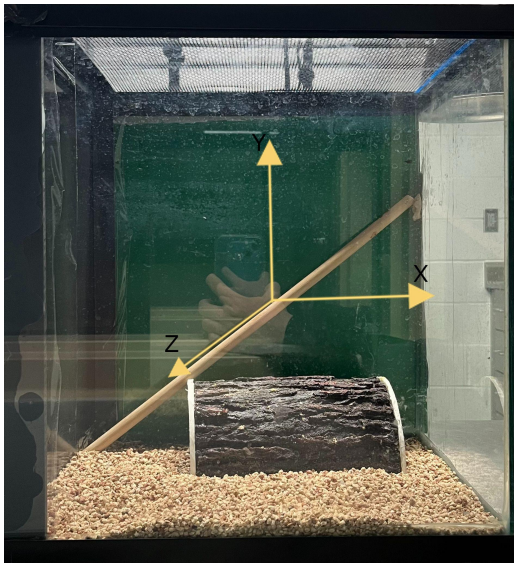

Fig. 3: Cage used to house lizards for the temperature experiments. The dimensions of the cage are indicated by the arrows. The cage is 32x38x41.2 cm, and sat 58.4 cm away from a two-way window so the Canon Vixia could record the lizards. Each cage had a heating lamp, UV light, perch, log, and substrate. The light and heating lamp were controlled by a BN-LINK 8 Outlet Surge Protector with Mechanical Timer.

To record the displays, we used a Canon Vixia Hf G21 HD camcorder, which has a frame rate of 60 fps. The camera was set on a tripod that would be adjusted based on the horizontal and vertical movement of the display lizard. Each trial was recorded for 20 minutes, starting from when an intruder lizard marked with Wite-Out was introduced to the cage. Each displaying lizard had their own respective intruder that was to be used for each trial (except for one experiment where one of the intruder's tails detached). Only the displays of the resident lizard were counted in the data analysis, and any displays by the intruder were ignored by the recorder. Jenssen's

(1977) definition of a headbob display was used to assess what constituted a display, which included lateral orientation and coupled movement of the head, legs, and tail. We considered two displays as separate when there was at least 30 seconds of no displaying in between each display. Any pause that lasted for less than 30 seconds was considered to be a part of a single display. Any displays that occurred more than 20 minutes after the intruder was introduced were not counted. Only one trial was done in a single day, with 1-4 days in between the next trial. The order of consecutive cold and warm trials was random for each lizard. After each lizard's last warm and cold trial, their body temperatures were recorded using a Fisher Scientific Traceable Expanded-range Thermometer, as well as the distance from the top of the orbit to the mouth of the displaying lizard (4mm). Room temperature was consistently measured towards the end of all of the experiments to account for any possible fluctuations in the temperature throughout the day.

For each 20 minute video we recorded of the lizards in either warm or cold conditions, we clipped all of the singular headbob displays from the resident. The data was analyzed using MATLAB R2021a Update 5 and the DLTdv8a video tracking application on a Mac desktop. In the DLTdv8a app we set the search area to 30, the tracker threshold to 8, and set the tracker to autoadvance. Then, we clicked five times on the upper edge of the eye, 5 times on the mouth line, and then once at a zero point on the perch or ground below the lizard. The difference between the mouth and eye was measured to be 4 mm. Then we switched from autoadvance to automatic, clicked the center of the eye, and let the frames run while the tracker recorded data points from the position of the eye during the headbob displays. Once the frames finished, we exported the x-y points, saved the file as 'flat,' and then analyzed the data in Microsoft Excel 2019. The Excel sheet was used to calculate the distance from the lizard's eye to the stable position perpendicular to the lizard's body, and the movement amplitude was converted to mm

using head height as a scale. Once we graphed the results on Microsoft Excel, we took the rise time (number of frames from bottom of a peak to the top of the peak) and amplitude (height of the peaks) of the first four normal peaks (depending on the efficiency of MATLAB and movement of the lizard). For further statistical analysis, we took the average of the four data points for both the rise time and amplitude for each clip and performed a one-way ANOVA using JMP 17.1.0. We also analyzed the difference between the number of responses for each trial using one-way ANOVA on JMP 17.1.0. We only analyzed two lizards because of difficulties with temperature control prior to the experiments using these two lizards. The temperature of the room fluctuated throughout the day, so we controlled it better for the two lizards we analyzed.

Stimulus Experiment 1: Rapid Movement Tests

The stimulus experiment took place over a period of 10 weeks, with 1-4 days in between each block of the experiment. We studied 10 different lizards that a were each housed in a small 19x19x24 cm box, with a hatch on the y-axis, a window on the z-axis, a perch horizontally across the x-axis of the cage, and faux foliage used for shade on top of a corn-cob substrate (Fig. 4). The 10 cages were all aligned next to each other on a table, each with its own light and heat sources. The room was kept at a temperature of 26.7℃, and the lizards were given 12 hours of light a day to mimic the natural environment.

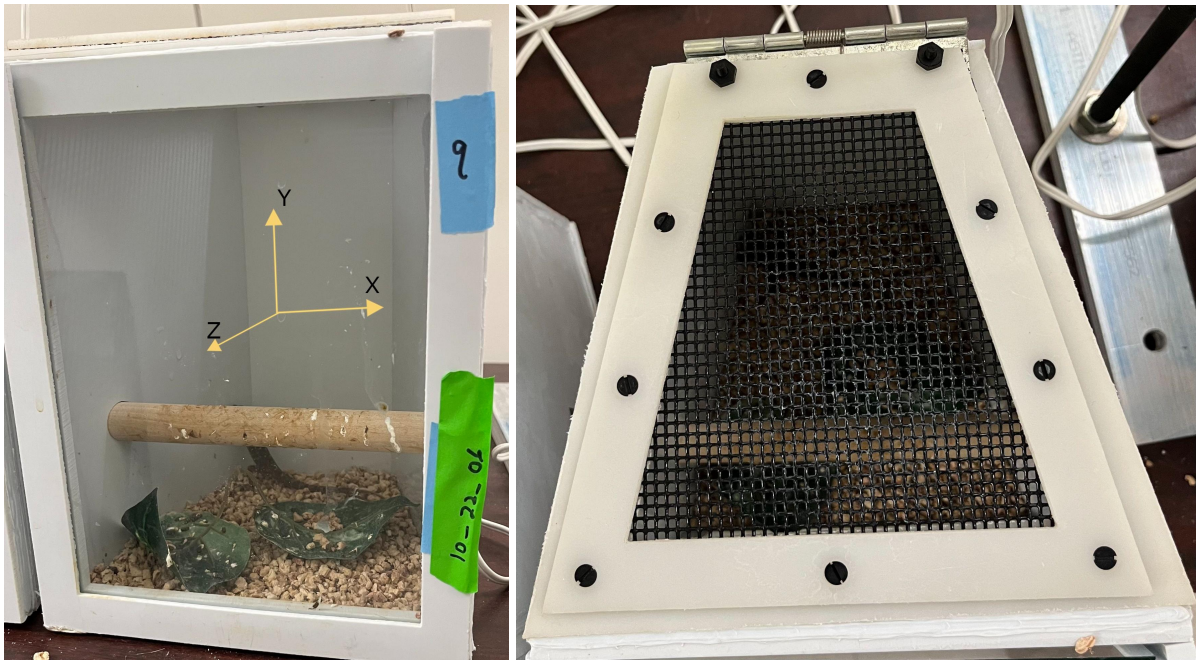

Fig. 4: Cage used to house lizards for the stimulus experiments. The lines indicate arbitrary axes assigned by me to help describe the physical nature of the cage. The cage was 19x19x24 cm. Each cage had the same organization of a rocky substrate, faux leaf, and a perch. Each cage also had its own heating lamp above the y-axis (not pictured).

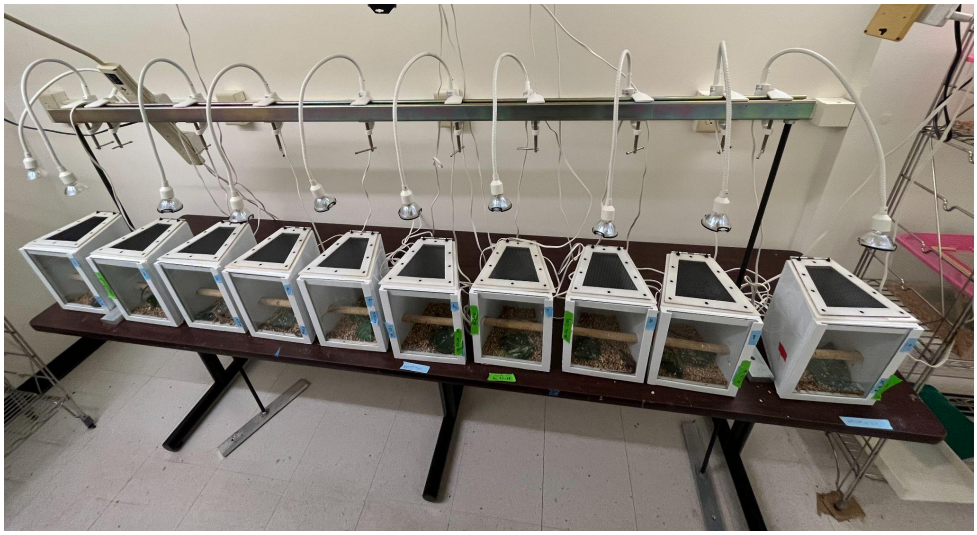

Fig. 5: The 10-cage setup used for both stimuli experiments. Each cage had the same setup (fig. 4), and housed one lizard for a ten week period (barring the lizard stayed alive). For each trial, we placed the oscilloscope 11.5cm from the window of the cage.

In order to create the stimulus, we used a 2007 Macbook laptop attached to a Powerlab 4/25 and a Wavetek Dual Hi/Lo Filter model 432 frequency filter set to 1 div/volt. The cutoff

frequency was set to 80Hz. The stimulus was a dot on a BK Precision 2190B Dual Trace Oscilloscope (100MHz) that moved based on the frequency and time inputs from the Powerlab and Wavetek frequency filter. The dot remained at the origin of the x-and-y-axis on the screen of the oscilloscope. The Powerlab set up the stimuli using Scope 4 v4.0.1. We set Input A of the Powerlab at 5V, and Input B at 10V. We also set the Time Base of 2kHz to have 1280 samples and a time of 500 ms. The first four seconds included the initial movement of the stimulus and it remained at an amplitude of 20 mm, with a reduction of 5 mm to mimic the natural bouncing of lizard headbob displays. The dot returned to its original position during the last second of stimulus activation. Each block contained five different trials at different frequencies: a control trial with no stimulus, a trial with a frequency of 20 Hz (45 ms), a trial with a frequency of 10 Hz (80 ms), a trial with a frequency of 5 Hz (150 ms), and a trial with a frequency of 2 Hz (310 ms) (Fig. 6). The order of each consecutive trial was randomized for each block. We conducted ten blocks throughout the experiment, with each lizard only being tested once per week to avoid habituation to the stimulus. A piece of cardboard was attached to the side of the screen of the oscilloscope in order to avoid any adjacent lizards from viewing the stimulus and habituating. The oscilloscope screen was positioned approximately 11.5 cm from the window of the cages. A positive response to the stimuli was indicated by a movement of the eye towards the stimulus. In order to view the response of the lizard, a Canon Vixia HF R52 digital camera was placed on a wheeled cart next to the oscilloscope, and the camera was attached to a GEChic portable monitor in order to see the video in the other room. The Powerlab, Wavetek, Macbook, and experimenter resided in a different room next to the room that contained the lizards to ensure the lizards did not see the experimenter. The visual angle was kept constant at an angle of 0.22° based on previous data on the ideal visual angle for *Anolis sagrei* (Fleishman 1986; Fleishman and Pallus

2010; Steinberg 2013). A piece of tape was put on the cart with the digital camera and oscilloscope to keep the visual angle constant. The experimenter would align the piece of tape to the eye of the lizard. If the lizard moved during the experiment, the experimenter had to enter the room and move the cart so the tape realigned with the lizard's eye. Before each block, the cart stayed in front of the lizard for three minutes without stimulus activation. After the initial three minutes, the trials started, with at least one minute in between each trial for the different frequencies. If the lizard reacted to the stimuli within four seconds, then the reaction was considered positive.

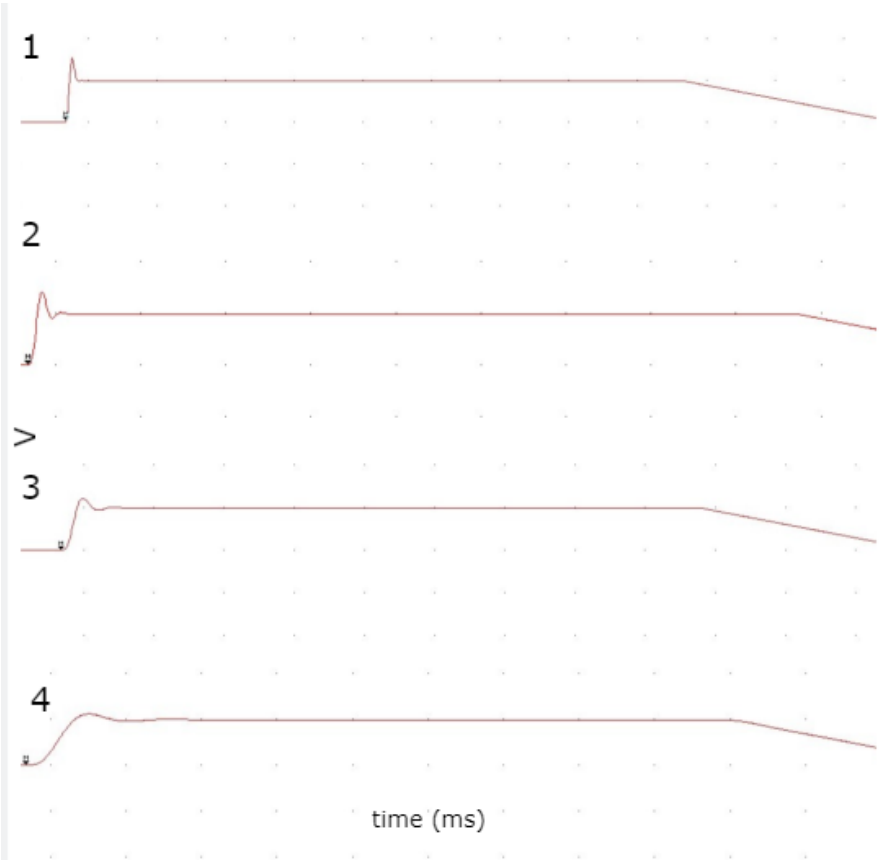

Fig. 6: Stimuli shape for the rapid movement experiment using Powerlab 4/15, a Dualtek frequency filter, and an oscilloscope. Stimulus 1 is 20 Hz (45 ms), stimulus 2 is 10 Hz (80), stimulus 3 is 5 Hz (150), and stimulus 4 is 2 Hz (310 ms). The y-axis represents voltage (V) and the x-axis represents time (ms).

We used JMP v17.1.0 to analyze the statistics of the stimulus experiments. To evaluate the differences in the reaction frequency for each stimulus amongst each lizard, we used a blocked one-way ANOVA, as well as a Tukey HSD test of the Logit-transformed data. We used a Logit transformation of the data since proportions do not follow a normal distribution, and the Logit would transform the data to be analyzed using practices for normally-distributed data. We looked at the Tukey HSD test of the data with the control trial (stimulus 5) and without the control trial. All of the data was analyzed using standard least squares.

Direction experiment

The direction experiment was very similar to the rapid movement experiment; it only differed by the type of stimulus we used. We used the same 2007 Macbook laptop attached to a Powerlab 4/25 and Wavetek Dual Hi/Lo Filter model 432 frequency filter to create and control the stimuli. We also used the same BK Precision 2190B Dual Trace Oscilloscope (100MHz) as the actual moving stimulus. We monitored the reaction of the lizard through the same set-up, with a Canon Vixia HF R52 set up next to the oscilloscope on a wheeled cart, and a GEChic portable monitor to watch the live video.

The lizards were kept in the same conditions as they were in the rapid movement experiment. Most of the lizards were changed between experiments and acclimated to the new environment. There were ten lizards present for the trials at any given time. Each trial tested five different stimuli. Stimulus 1 was a direction-changing stimulus with two direction-changing elements, one with a rising amplitude of 2V and the other with a falling amplitude of -1 V, a rise time of 82 ms, and a fall time of 85 ms; stimulus 2 was a square wave stimulus with an amplitude up to 1 V, a rise time of 88 ms and a fall of 89 ms; stimulus 3 was a square wave

stimulus with an amplitude of 2 V, a rise time of 80 ms, and a fall time of 80 ms; stimulus 4 was a square wave with an amplitude of 1 V, a rise time of 45 ms, and a fall time of 42 ms; trial 5 was a control trial and consisted of no moving stimulus (Fig. 7). There were ten blocks of these trials, and the order of each trial was randomized. Unlike the rapid movement experiment, the stimulus duration only lasted based on how fast the stimulus was. If the lizard moved its position during a trial, then the experimenter moved the wheeled cart to align the oscilloscope with the eye of the lizard to maintain the 0.22° visual angle. We set up the oscilloscope in front of the lizard for at least 3 minutes before each block, and then waited at least 1 minute before each trial. We counted a positive reaction as any change in eye or head position towards the direction of the moving stimulus for a duration of four seconds. We gave the lizards at least 5-8 days between each block to prevent acclimation to the stimuli.

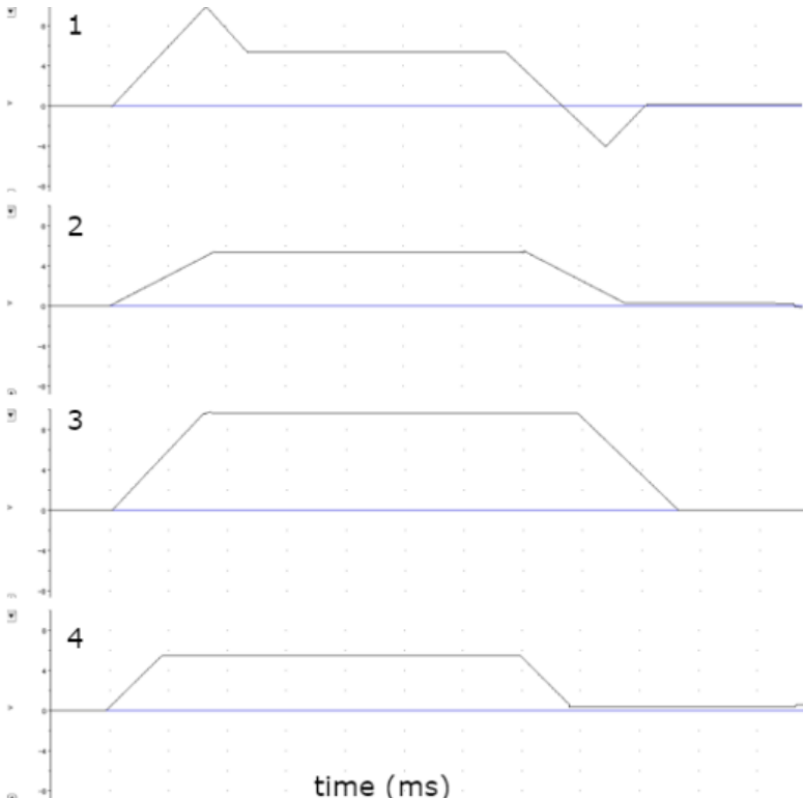

Fig. 7: Stimulus types for the direction-changing experiment. Stimulus 1 represents the direction-changing stimulus with a rise time of 82 ms and a fall time of 85 ms; stimulus 2

represents a squarewave with a lower amplitude, a rise time of 88 ms, and a fall time of 89 ms; stimulus 3 represents a squarewave with a higher amplitude, a rise time of 80 ms, and a fall time of 80 ms; stimulus 4 represents a rapid square wave with a rise time of 45 ms and a fall time of 42 ms. Stimuli were made using Powerlab 4/15, a Dualtek frequency filter, and a BK Precision 2190B Dual Trace Oscilloscope (100MHz).

We used JMP v17.1.0 to analyze the statistics of the stimulus experiments. To evaluate the differences in the reaction frequency for each stimulus amongst each lizard, we used a blocked one-way ANOVA, as well as a Tukey HSD test of the Logit-transformed data. We used a Logit transformation of the data since proportions do not follow a normal distribution, and the Logit would transform the data to be analyzed using practices for normally-distributed data. We looked at the Tukey HSD test of the data with the control trial (stimulus 5) and without the control trial. All of the data was analyzed using standard least squares.

Muscle Stimulus Experiment

In order to further investigate whether the muscles of *A. sagrei* intrinsically bounce to produce the direction-changing effect, we isolated and stimulated muscle to graph the DAP. We anesthetized the lizard before euthanizing him and isolating the gastrocnemius muscle and thigh, which was hoisted up for stimulation. We stimulated the muscle using electrodes pierced through the thigh muscles above the gastrocnemius. To create the stimulus, we used an LE 12106 digital pulse stimulator and used a voltage and pulse frequency to induce tetanus (70 points per second). We then filmed the muscle contraction using a Canon Vixia Hf G21 HD camcorder (60fps). We set up a solid background behind the muscle so the camera could focus on the muscle easier. We added a piece of blue clay at the end of the toe so the video analyzer had a focal point to track the movement of the leg.

We analyzed the movement of the leg using MATLAB R2021a Update 5 and the DLTdv8a video tracking application on a Mac desktop. We set the tracker threshold to 8 and set the search area to 12. We used the auto-advance mode, and then clicked the bottom of the clay ball five times, the top of the clay ball five times, and the bottom of the forceps holding the muscle five times (used as a stable position). Then, we changed the mode to automatic and let the tracker run until the frames finished. We took the x-y points (saved as flat, not sparse) from the video tracker app and graphed them using Microsoft Excel 2019. The result was a DAP of the muscle contractions.

Results

Temperature Experiment

We analyzed 11 displays in cold conditions, and 13 displays in warm conditions. The lizards in the cold temperature ranged from 27-30℃, and the lizards in the warm temperature ranged from 31-35℃. The average room temperature was around 22℃, but ranged from 21-24℃. Figure 8 shows the bar graphs of the effects of temperature on amplitude (a) and rise time (b). There was a slight difference in the averages for both rise time and amplitude in the different temperature conditions (Fig 8), but there was no significant difference ($p=0.8744$ for amplitude; $p= 0.1465$ for rise time).

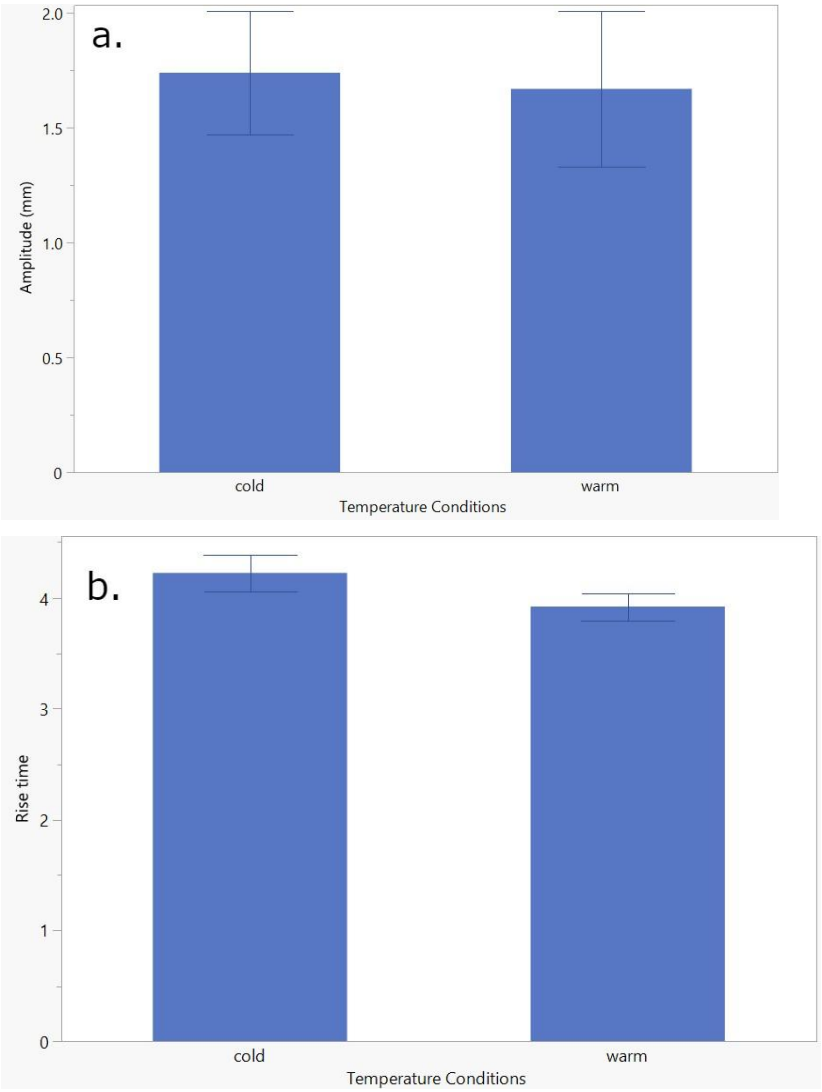

Fig. 8: Effects of temperature on amplitude (mm) and rise time (frames) of *Anolis sagrei* assertive headbob displays. The graphs above show the different temperature conditions vs. amplitude (mm) (a.) and rise time (frames) (b.). a) The average amplitude in cold temperatures (n= 11) was 1.743 mm, and the average amplitude in the warm temperatures (n= 13) was 1.672 mm. b) The average rise time in cold conditions was 4.227, and the average rise time in the warm temperatures was 3.923. There was no significant difference for either amplitude or rise time based on temperature (p=0.8744; p=0.1465). Standard error bars shown.

We also looked at the frequency of displays between the different temperature conditions (Fig. 9). We only looked at the two lizards after the temperature conditions were more controlled. We found that there was a significant difference between the temperature conditions for the

number of displays (p=0.0403). The lizards displayed more in warmer conditions than colder conditions on average.

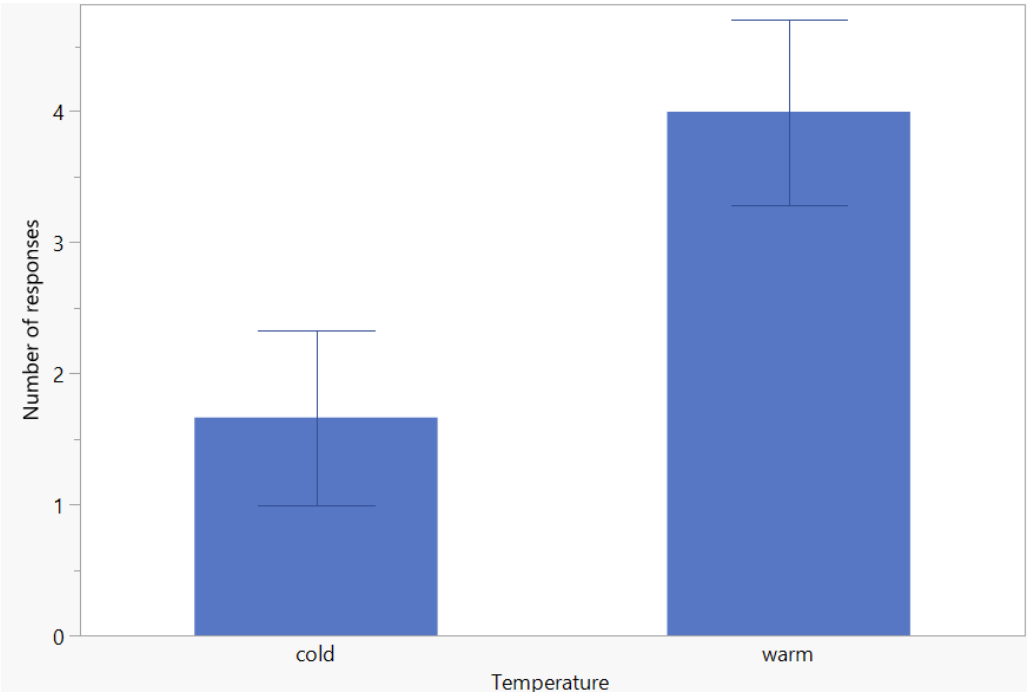

Fig. 9: The frequency of headbob displays between different temperature conditions. We compared the frequency between two different lizards, and each lizard had at least three trials of each temperature condition. We analyzed a total of 6 cold trials and 5 warm trials, each of which had a varying degree of individual displays. The bars display the average number of responses for the cold temperature (mean= 1.667) and the warm temperatures (mean= 4.0). There was a significant difference in the number of responses between the trials (p=0.0403). Standard error bars are shown.

We also analyzed the DAPs to look for any patterns based on temperature. The DAPs had a consistent early pattern of 2-3 headbobs and then a headbob with a longer plateau immediately followed by a quick spike. It was consistent in both warm and cold conditions, likely indicating it is a part of the core display. The headbobs after the quick spike were less stereotyped and differed from display to display. The warmer displays also had more variability in their amplitude (figure 8a).

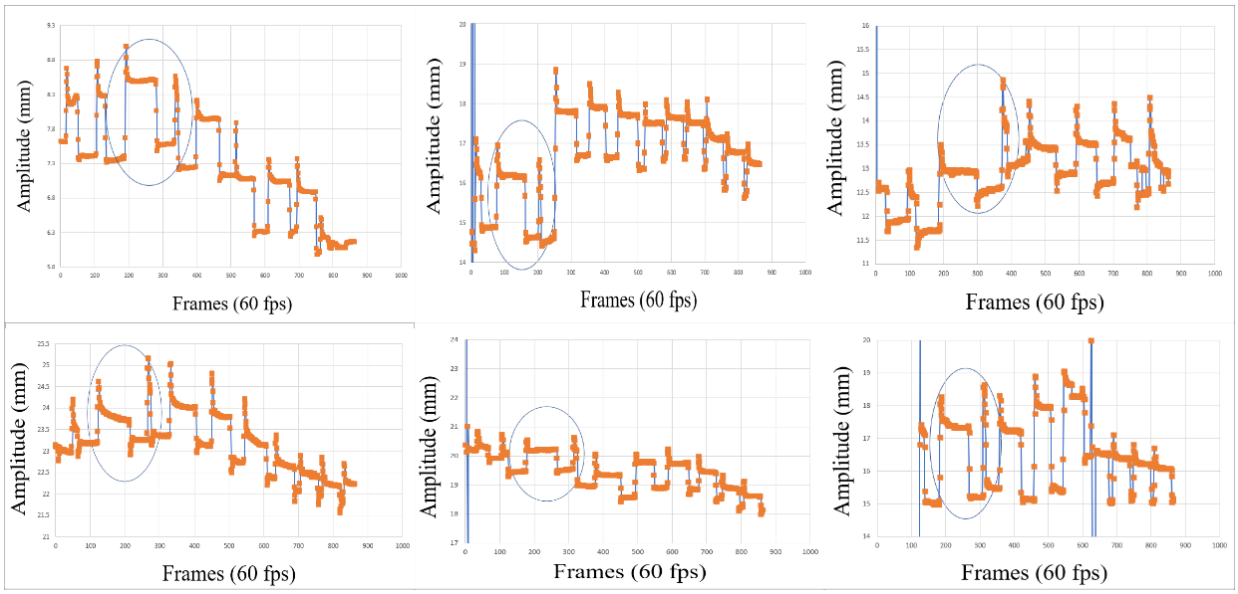

Fig. 10: Common patterns of *A. sagrei* DAPs in cold and warm conditions. DAPs were captured using a Canon Vixia Hf G21 HD camcorder and analyzed using MATLAB and Microsoft Excel. The top row (left to right) represents cold displays. The bottom row (left to right) represents warm displays. The circled area represents the commonality between the displays. The x-axis represents the amplitude of the head (mm). The y-axis represents the frames at a frame rate of 60 fps.

Rapid Movement Experiment

We analyzed the results for the 10 blocks of 5 trials for the 10 lizards, even if we had to replace the lizard during the experiment. We continued to analyze the results as if we never replaced the lizard. Figure 11 shows the average proportion of positive response from all of the lizards for each trial type. The averages of trials 1-4 were all fairly similar, falling within a range of 0.08, but they all differed significantly from trial 5 (mean=0.11) ($p=0.0001$). To analyze the results, we performed a blocked paired ANOVA, as well as a pairwise Tukey HSD test of the Logit transform proportion of positive response. Trials 1-4 did not significantly differ from each other ($p=0.64$), so none of those stimuli were more likely to cause a response from any given lizard.

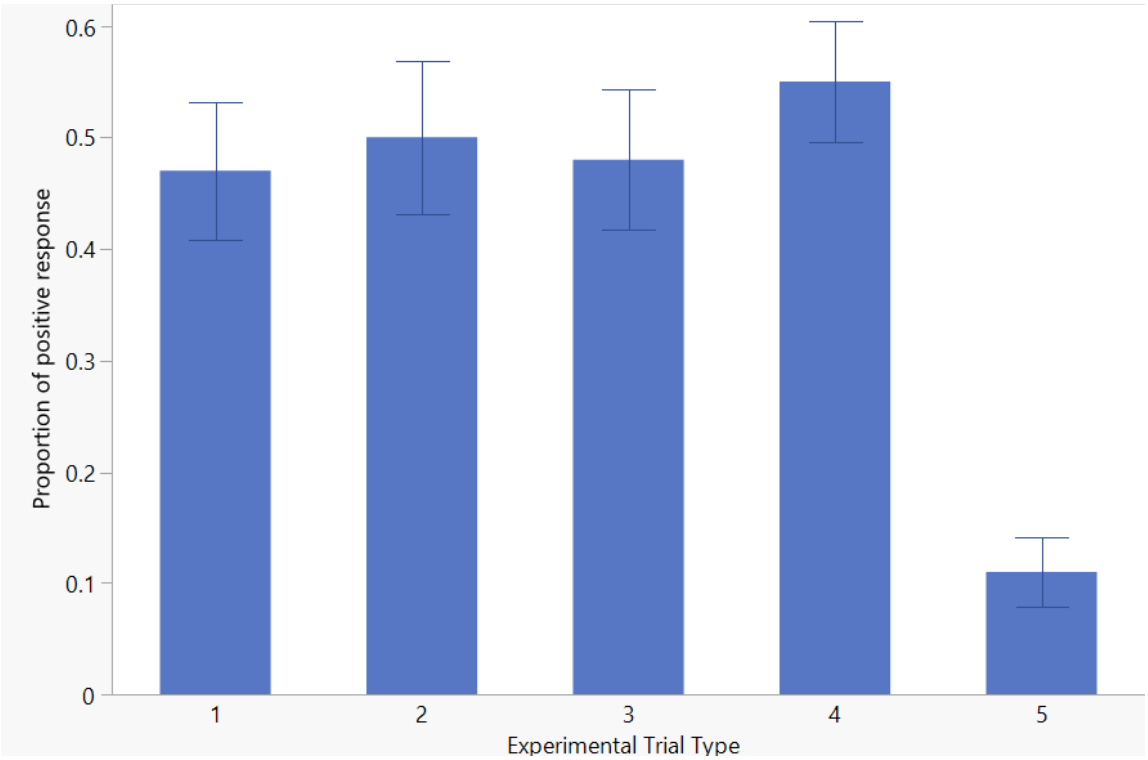

Fig. 11: The proportion of positive responses of ten different lizards to different motion stimuli based on speed and amplitude. We conducted 500 different trials, with four different motion stimuli and one control trial, used across ten lizards for ten weekly blocks. Trial 4 had the highest average proportion of response (mean=0.55), followed by trial 2 (mean=0.50), trial 3 (mean=0.48), trial 1 (mean=0.47), and then trial 5 (mean=0.11). Trials 1-4 differed significantly from trial 5 in terms of positive mean response (p=0.0001), but they did not differ significantly from each other (p=0.64). Standard error bars are shown.

Direction-changing Experiment

We analyzed the results for the 10 blocks of 5 trials for the 10 lizards, even if we had to replace the lizard during the experiment. We continued to analyze the results as if we never replaced the lizard. Figure 12 shows the average proportion of response for each trial type. The graph shows the averages between all lizards instead of representing the differences among an individual lizard's responses. The average number of responses differs based on our hypothesis. The average proportion of response for trial 1 was 0.520; for trial 2 was 0.360; for trial 3 was 0.400; for trial 4 was 0.470; and trial 5 was 0.150. To analyze whether these differences were

significant, we conducted an all pairwise differences Tukey HSD test and a blocked oneway ANOVA of the Logit transform of the proportion response. The results indicate that all trial types were significantly different from the control (trial 5), but the lizards did not significantly react more to one stimulus than the other for trials 1-4.

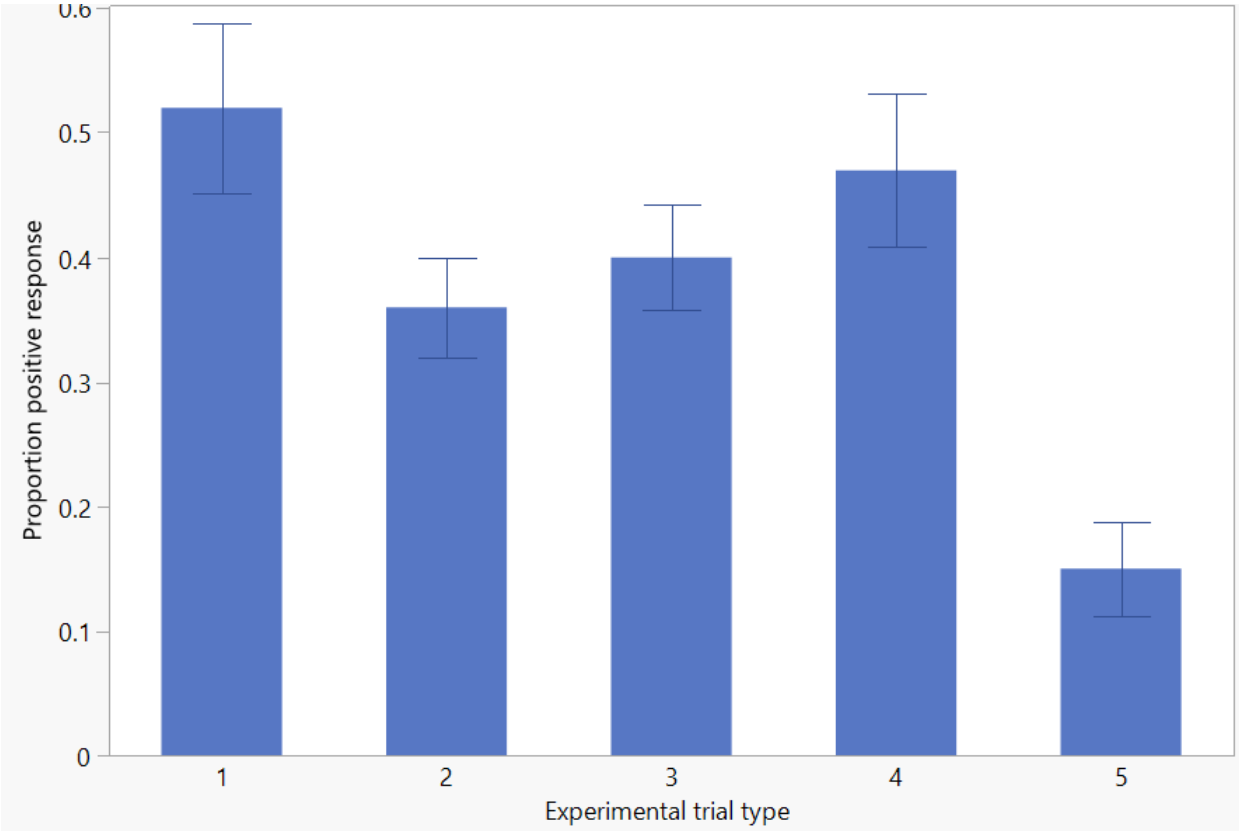

Fig. 12: The proportion of positive response of 10 different lizards using different motion-stimuli and direction-changing elements. We conducted 500 different trials, with four different motion stimuli and one control trial, used across ten lizards for ten weekly blocks. Trial type 1 had the highest mean positive response (mean=0.520), followed by trial type 4 (mean=0.47), trial type 3 (mean=0.40), trial type 2 (mean=0.36), and then trial type 5 (mean=0.15). The response to trials 1-4 was significantly different from trial 5 (p=0.0001), but they did not significantly differ from each other (p=0.1432). Error bars are shown.

Muscle Stimulus Experiment

The DAP of the isolated muscle that was stimulated by the electrodes showed no direction-changing elements (Figure 13). There is no reverse motion of the muscle during contraction.

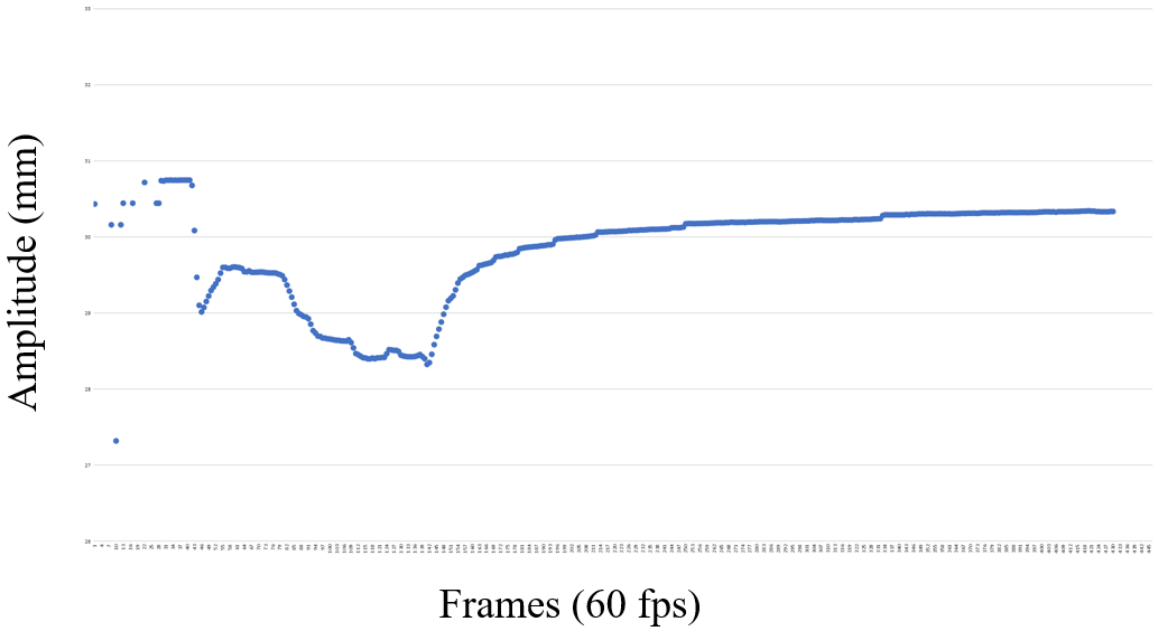

Fig. 13: DAP of the isolated gastrocnemius muscle using an electrode stimulus. The gastrocnemius muscle of *A. sagrei* was isolated and stimulated using 70 points per second. The movement was recorded using a Canon Vixia Hf G21 HD camcorder and analyzed using MATLAB. The x-axis represents the number of frames at a frame rate of 60 fps. The y-axis represents the amplitude of the muscle movement (mm).

Discussion

Temperature Experiments

Our results indicated that there was no significant difference between cold and warm conditions on the effectiveness of the headbob display. The amplitudes and rise times of displays did not significantly differ for either condition. This means our experiment did not support our hypothesis that temperature should have an effect on the efficiency of the headbob display. In terms of honest signaling, that means the temperatures that we used in the experiment do not have an effect on the aggressiveness of the signaler, and thus the onlooker does not see them as a worse fighter. This is further supported by our rapid stimulus experiment, which indicated that the onlookers could not sense a difference between the different stimuli based on varying speeds and amplitudes. Therefore, the temperature differences that we used had no effect on the

aggressiveness of the headbob signal, and did not change the perception of the signaler's ability to fight. The honest signaling aspect was not affected in the way that we hypothesized. However, temperature did have an effect on the frequency of signaling of the headbob displays. The lizards displayed significantly more in warmer temperatures than they did in colder conditions. This has an interesting dynamic with the previous result, since this result does indicate that an aspect of the signal information changes with differences in temperature. The differences in frequency indicate that the muscular function of *A. sagrei* is affected based on the temperature, which previous research has already indicated, but the efficiency of the display is not (Bennett 1985; Lailvaux & Irschick 2007). The temporal pacing of the display was not affected, which signals the aggressiveness of a lizard. Even though temperature did affect their muscles in terms of frequency, they expended effort to maintain the temporal pacing of warm displays. This suggests that the lizards prioritized the temporal pacing to not appear as worse fighters. Differences in amplitude and speed would make the lizards seem like worse fighters, but what is the effect of frequency on how onlookers view signalers? This study did not explore this question, which makes an interesting topic to study in the future. A question also arises about whether we can predict one lizard is less likely to display in the cold compared to another. Does the bigger and more dominant lizard in a dispute always display more frequently in the cold than a weaker and smaller one? Or is it an unpredictable phenomenon that does not relate to the honesty of a signal? This also has an implication for the effects of climate change. Drastic changes in temperature, as well as an increase in the frequency of tropical storms, could have a significant impact on the signal efficacy of *A. sagrei*, and likely all anoles.

Even though our experiment did not support our hypothesis, it does not mean that our hypothesis is incorrect. There were many aspects of both the temperature experiment and rapid

eye stimulus experiment that are important to take into account when looking at the data and results. The room temperature in the temperature experiment was not kept consistent throughout the day. This meant that even though we looked at five different lizards for the experiment, we could only analyze two of them. The room temperature changes were too much of a confounding variable to include the results from the first three lizards in our analysis. This greatly restricted our sample size, especially since one of the two lizards we analyzed did not display often in the cold conditions. The lizards were also very effective at basking under the UV light. They could get their body temperatures to almost the same temperature as the warm conditions, differing by only 1℃ in some instances. This difference is not large enough to justify considering one cold condition and one warm condition. However, if we made the temperature differences too large, we would not have enough displays in the cold conditions since they display less frequently in the colder conditions. This makes it very difficult to create a cold condition that has a significant difference from the warmer condition, while also maintaining a high enough temperature where displays occur. So, the experimental conditions were not perfectly controlled and there is room for improvement for any future experiments looking at this hypothesis.

Direction-changing Experiment

Our results for the direction-changing stimulus experiment indicated that there is no significant difference between direction-changing and non-drection-changing motion stimuli. However, the direction reversing stimulus did receive the most responses, although due to variation in the response rates it was not responded to significantly more often than the other stimuli. This means that our experiment did not support our hypothesis that the direction-changing element at the beginning of each headbob was an evolutionary adaptation to

increase the detectability and temporal precision (to the viewer) of the display, rather than an artifact of muscle physiology. We suspected that the abrupt reversal of direction in the display movements might have been due to elastic rebound of the muscle after abrupt movements. However, the muscle stimulus experiment showed that when we stimulated the gastrocnemius muscle to induce tetanus and contract rapidly, the muscle did not produce the direction-changing element. This suggests that it is not part of the muscle physiology, and there needs to be another aspect involved to explain the direction-changing element. These two conflicting results make it difficult to fully understand the role of the direction-changing element in the displays. Restrictions in the motion stimulus experiment, on top of the results from the muscle stimulation experiment, led us to believe that our hypothesis was actually supported and the direction-changing element is an evolutionary adaptation and an active process.

The raw data from the motion stimulus experiment indicates a trend which corroborates our hypothesis (figure 12). The highest proportion of response occurred with the direction-changing element based on average, and the error bars do not overlap with the second or third trial stimuli. However, the statistics suggest that there is no significant difference between stimuli 1-4. This was surprising because the error bars did not overlap, and there is a trend present in the data. We believe that if we increased the sample size, we would see a significantly greater response to the direction-changing stimulus. The sample size was fairly small, especially when we consider the variability in the response of the lizards. We controlled for the reactability by conducting a blocked oneway ANOVA, but controlling for this does little when 1-3 lizards of the total 10 in the sample have low reactability to all stimuli. This does not indicate that they see no significant difference between motion stimuli and no stimuli (supported by the significant difference between trials 1-4 and trial 5). It just means some lizards are not as

reactive to our motion stimulus. This shrinks the sample size even more. If we included more reactable lizards and/or a larger number of lizards, then we could improve the quality of the data. This does not guarantee that our hypothesis would be supported, but it would provide more confidence in the results. Another variable to consider is that these are not domesticated or captive-bred animals; they are afraid of humans and do not easily cooperate. Placing the oscilloscope in front of their eye was a challenge most of the time, since they would hide or move when I came into the room. We tried our best to maintain a visual angle that was most likely to elicit a reaction from the lizards, but it was not guaranteed that they could see the dot at all times. If they were not on the perch or if their bodies were oriented towards the oscilloscope, it was more difficult to discern if they could actually see the stimulus. We believe that they could since reactions were not uncommon in these positions, but it is unknown whether this has an effect on reactability. So, finding a more reliable way to sense whether the lizards can see the stimulus would also provide more confidence to our data.

One future research question we would like to explore is these same experiments except utilizing a species where the direction-changing element is not present in the display, such as *A. carolinensis*. We are curious to see if the trend in figure 12 is also present in this species, and what the muscle stimulation shows. We would expect the muscle to move in a very similar way if it does not have the direction-changing element, but we would expect a smaller gap in between trials 1-4 for the motion stimulus experiment. This would also provide us with more confidence in our hypothesis and muscle stimulation results.

Conclusion

The honest signaling of the anoline headbob display was a really interesting topic to explore, and was very easily manipulated since motion is a simple stimulus. Our hypothesis on the effects of temperature on the headbob display may not have been supported by our experiments, but better control of many of the variables, as well as a larger sample size, would produce more accurate results of the trend. Since temperature does have an effect on display signaling (i.e., frequency), then it is not unlikely that the information of the signal is also affected. The results of the direction-changing experiments are not completely clear, but we are more inclined to believe that our results are consistent with our hypothesis. Increasing sample sizes, as well as a comparative study with other *Anolis* species would provide us with a clearer idea of the purpose of this element in *A. sagrei*.

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