

AN ABSTRACT FOR THE THESIS OF

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A lizard that sees a predator sometimes becomes immobile to avoid detection, but it is unclear how the energy demands of this strategy compare to those of a calm lizard. I quantified the metabolic response of adult male *Anolis carolinensis* when in visual contact with a predator and with prey. Lizards' oxygen consumption when seeing prey was not significantly different from baseline oxygen consumption levels ($P = 0.416$), but lizards reduced oxygen consumption when seeing a predator ($P < 0.001$). Lizards made fewer body movements and changes of gaze when seeing a predator than when seeing prey, consistent with the antipredator strategy of reducing movement to minimize the likelihood of drawing a predator's attention.

Metabolic Response to Visual Contact with Predator and Prey in the Green Anole (*Anolis
carolinensis*)

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Preface

This thesis was prepared following the publication style of the Journal of Herpetology. Research was conducted in accordance with the Emporia State University Animal Care and Use Committee (Approval Number: 11-005), and followed the American Society of Ichthyologists and Herpetologists (ASIH), Society for the Study of Amphibians and Reptiles (SSAR), and the Herpetologists' League (HL) guidelines for use of reptiles in field and laboratory research (HACC, 2004).

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FIG 2 – Mean oxygen consumption measured in $\mu\text{l O}_2 \cdot \text{g}^{-1} \text{ lizard mass} \cdot \text{min}^{-1} \pm \text{SE}$ during prey trials. Oxygen consumption was measured before the trial (“baseline”), while each anole could see a chamber containing insect prey (“crickets”), and after the chamber was covered (“post-crickets”). $n = 13$.

FIG 3 – Mean oxygen consumption measured in $\mu\text{l O}_2 \cdot \text{g}^{-1} \text{ lizard mass} \cdot \text{min}^{-1} \pm \text{SE}$ during predator trials. Oxygen consumption was measured before the trial (“baseline”), while each anole could see a chamber containing a predator (“snake”), and after the chamber was covered (“post-snake”). $n = 13$.

FIG 4 – Mean number of body movements per min. during the 10 minute interval when an anole could view a cornsnake (“predator”), insects (“prey”), or an empty chamber (“control”). Vertical bars are $\pm \text{SE}$. $n = 12$.

FIG 5 – Mean number of gaze changes per min. during the 10 minute interval when an anole could view a cornsnake (“predator”), insects (“prey”), or an empty chamber (“control”). Vertical bars are \pm SE. $n = 12$.

Introduction

Lizards use several antipredator strategies, including fleeing to escape a predator, signaling to the predator that a surprise attack is no longer possible, and immobility (Lima and Dill, 1989). Immobility involves minimizing movement; Labra and Leonard (1999) found that when lizards in the genus *Liolaemus* became immobile in response to a model of a bird predator, the lizards ceased activity and reduced their breathing rate and did not immediately resume activity after the model was removed. This reduction in breathing rate could be an attempt to reduce movement that might draw the attention of a bird watching from above.

Immobility is commonly used by well-camouflaged lizard species (Lima and Dill, 1989), including anoles (Heatwole, 1968; Leal and Rodríguez-Robles, 1995, 1997). The degree to which a species relies on immobility is positively correlated with the species' ability to use camouflage (Heatwole, 1968). The length of time in which an individual lizard relies on immobility varies with circumstances; if the predator has not yet seen the lizard, immobility reduces the chance of drawing the predator's attention, and if fleeing would cause the lizard to lose sight of the predator, immobility is safer (Cooper, 1997).

The metabolic cost of immobility is unclear. Does fear increase aerobic metabolism above baseline levels, or does the reduced movement decrease aerobic metabolism? To determine this aspect of the metabolic effect of immobility, I measured oxygen consumption in male Green Anoles (*Anolis carolinensis*) looking at a Cornsnake (*Pantherophis guttatus*).

Oxygen consumption is commonly used to measure metabolic cost. Cells need ATP to function, and ATP production requires oxygen during aerobic metabolism (Hill et

al., 2008). An increase in energy use causes an increase in oxygen demand and thus an increase in oxygen intake if the animal is to rely totally on aerobic respiration (Pough and Andrews, 1985). If immobile lizards increased oxygen consumption in response to the snake, I would conclude that immobility is a costly strategy in terms of aerobic respiration, possibly due to costs associated with tensed muscles in anticipation of fleeing. If they decreased oxygen consumption, I would conclude that the reduced movement and possibly reduced breathing rate lowered the aerobic cost of immobility below baseline energy use.

Snakes are major predators of anoles (e.g. Henderson, 1984), and some snakes eat *A. carolinensis* (Orleb, 1951; Savidge, 1988). Cornsnakes are sympatric with *A. carolinensis* (Mitchell, 1977) and have been reported to eat lizards (Resmer, 1999), so Green Anoles should recognize a cornsnake as a predator.

I measured oxygen consumption of lizards looking at house crickets (*Acheta domestica*) to compare the effect of immobility to that of another situation in which a lizard might use energy. These anoles were maintained on house crickets, as is common for anoles in the lab (e.g., Stanger-Hall et al., 2001), and they recognized house crickets as prey (pers. obs.). I expected prey recognition to cause anoles to expend energy, as Green Anoles occasionally attempt to move through a clear plastic barrier to reach crickets (pers. obs.). I compared oxygen consumption in these trials to oxygen consumption in predator trials. I expected greater oxygen consumption in prey trials due to the presumed cost of attempts at prey capture and presumed lower cost of immobility as an antipredator strategy. I also conducted behavioral observations under the same conditions to determine whether Green Anoles would alter their behavior in ways that

corresponded with changes in oxygen consumption. I hypothesized that the anoles would make fewer movements in the presence of a predator than in the presence of prey or an empty chamber, likely to minimize the chance of drawing the predator's attention.

Materials and Methods

I purchased 13 male *A. carolinensis* from Reptmart.com. Their mean mass was 4.9 g, ranging from 3 to 6 g. Lizards were each kept in an individual Reptibreeze cage (base 40.5 x 40.5 cm, 51.0 cm tall) with newspaper substrate, a plastic mesh hammock for basking, fake branches made of rolled-up newspaper, and a small potted plant (Golden Pothos, *Epipremnum aureum*). Each pair of cages shared a 75 W basking light (ZooMed) on a 12L:12D photoperiod. Cardboard partitions separated each cage, preventing visual contact between neighbors. Lizards were each fed two mid-sized house crickets every two days. Cages were misted with water daily and each cage had a water dish.

Oxygen Consumption Trials-

Trials took place inside a lidless cardboard box (base 48.0 x 48.0 cm, 27.0 cm tall) so the lizard could not see the lab or movement in the lab. A clear plastic stimulus chamber (base 20.0 x 13.0 cm, 16.0 cm tall) contained a cornsnake or crickets or nothing and was covered by a towel. I placed a lizard in a glass test flask (3.5 x 3.5 x 13.5 cm) that was flat on the side facing the plastic chamber and I allowed the lizard to habituate to establish baseline oxygen consumption. I used a Qubit Systems S102 Flow-Through Oxygen Sensor with LoggerPro software (Qubit Systems, Kingston, Ontario, Canada) to record percent oxygen in the flask once per second.

Once the lizard's oxygen consumption was constant ($\pm 0.01\%$ oxygen) for at least 20 minutes, I removed the towel from the plastic stimulus chamber and allowed the lizard to see inside the chamber for 10 minutes. During predator trials a medium-sized wildtype color cornsnake (200 g, 96 cm long) was in this chamber; during prey trials six crickets (approx. 2 g total mass) were in this chamber, and during control trials this chamber was empty. In all trials the plastic chamber sat against a white background to enhance visibility of its contents. For these trials, $n = 13$. Each anole experienced the trials in random order (corrected so that one third of the lizards experienced a given stimulus first).

I recorded lizard oxygen consumption and noted (but did not quantify) lizard behavior during the 10 minute trial, then replaced the towel so that the stimulus chamber was hidden. I then recorded oxygen consumption and noted behavior for the next 10 minutes to determine the lizard's immediate reaction to losing visual contact with the predator or prey. Prior to any experiments, lizards were habituated to being in the flask for 20 min daily for one week; the first few times a lizard was in the flask, it would close its eyes and not respond to any stimulus, and habituation replaced this behavior with exploratory behavior. All trials were conducted between 23 July 2011 and 17 August 2011 between 0800 h and 1200 h during the lizards' normal activity period; light conditions were normal and temperature ranged from 20.3 °C to 23.6 °C.

Percent of oxygen present in the jar was converted to volume of oxygen consumed, which was then converted to standard temperature and pressure (Qubit Systems, 2006). I used one-way analyses of variance (ANOVA) for all three stimuli to compare baseline, treatment, and post-treatment oxygen consumption. Where significant

differences existed, I used paired t-tests to compare baseline to treatment oxygen consumption and treatment to post-treatment oxygen consumption.

Behavioral Trials-

I placed the plastic chamber used in the previous experiment covered by a towel into the lizards' home cages. The chamber was covered for 3 min, then uncovered for 10 min. I recorded body movements (e.g. step forward, step to the side, jump) and number of gaze changes (changing from looking with one eye to looking with two eyes or changing from one eye to the other eye) during the three parts of each trial. Predator trials had the same snake in the chamber, prey trials had six mid-sized crickets in the chamber, and control trials had nothing in the chamber. For each category of trial, $n = 12$. All trials were conducted between 16 September 2011 and 19 September 2011 between 0800 h and 1200 h within the lizards' normal activity period. Data were analyzed by two-factor ANOVA comparing stimulus and trial stage (baseline, treatment, post-treatment). Each lizard had at least one day in between trials and experienced trials in random order (corrected so that one third of the lizards experienced a given stimulus first).

Results

Oxygen Consumption Trials-

Mean oxygen consumption among baseline, treatment, and post-treatment times did not vary significantly in control trials ($F_{2,36} = 0.17$, $P = 0.841$; Fig. 1), nor did they vary significantly in prey trials ($F_{2,36} = 0.90$, $P = 0.416$; Fig. 2), but there was significant variation in predator trials ($F_{2,36} = 15.47$, $P < 0.001$; Fig. 3). Paired t-tests for predator trials showed a significant difference between baseline and treatment levels ($t = 5.80$, $P < 0.001$, $df = 12$) and no significant difference between treatment and post-treatment ($t =$

0.44, $P = 0.671$, $df = 12$). The change in oxygen consumption during predator trials tended to increase with increasing snake movement, but this was not quantified. Single-factor ANOVA showed no significant difference among baseline values for predator, prey, and control trials ($F_{2,36} = 0.61$, $P = 0.550$).

Lizard behavior was noted but not quantified during these trials. While establishing baseline oxygen consumption values, lizards turned around in the flask and pushed their snout against flask surfaces. In control trials, this exploratory behavior continued during treatment and post-treatment periods. During the treatment period of prey trials, some lizards turned their head to track cricket movements between bouts of lizard exploratory behavior, whereas others ignored crickets and continued with exploratory behaviors; lizards continued or resumed exploratory behaviors in the post-treatment period. In predator trials, lizards showed exploratory behaviors during the baseline period but became immobile and tracked snake movements only with one eye during treatment periods. Lizards maintained this immobility and focused on the chamber during the post-treatment period; no lizard resumed exploratory behavior. All lizards were bright green during all trials, despite a brown background.

Behavioral Trials: Body Movements per Minute-

Two-factor ANOVA showed that stimulus (predator, prey, control) had a significant effect on body movements per minute ($F_{2,99} = 4.79$, $P = 0.010$), but that trial stage (baseline, treatment, post-treatment) did not ($F_{2,99} = 0.06$, $P = 0.938$). There was no interaction between stimulus and trial stage ($F_{4,99} = 0.84$, $P = 0.500$). Because stimulus was significant and trial stage was not, I compared the number of body movements during the stimulus stage of all trial types using single-factor ANOVA, which showed

significant differences among the three stimuli ($F_{2,33} = 3.88$, $P = 0.031$). Paired t-tests showed a significant difference in the number of movements when anoles were exposed to a predator or prey ($t = 2.55$, $P = 0.027$, $df = 11$) and between when anoles could see a predator or an empty chamber ($t = 2.63$, $P = 0.023$, $df = 11$), but no significant difference occurred between prey and control trials ($t = 0.91$, $P = 0.380$, $df = 11$). Anoles made significantly fewer body movements in predator trials than in other trials (Fig. 4).

Behavioral Trials: Gaze Changes per Minute-

Two-factor ANOVA on the number of gaze changes per minute showed significant differences based on stimulus ($F_{2,99} = 7.98$, $P < 0.001$), no significant differences based on trial stage ($F_{2,99} = 0.00$, $P = 0.999$), and no interaction between stimulus and trial stage ($F_{4,99} = 0.15$, $P = 0.963$). Single-factor ANOVA comparing the number of gaze changes per minute during stimulus periods showed significant differences among the three types of trials ($F_{2,33} = 4.47$, $P = 0.019$). Paired t-tests showed significant differences in the number of gaze changes per minute when anoles viewed a predator or prey ($t = 2.46$, $P = 0.032$, $df = 11$) and when anoles viewed a predator or an empty chamber ($t = 2.66$, $P = 0.022$, $df = 11$), but no significant difference occurred between prey and control trials ($t = 0.76$, $P = 0.464$, $df = 11$). Anoles made significantly fewer gaze changes in predator trials than in other trials (Fig. 5).

Discussion

Oxygen Consumption-

The lack of significant difference between baseline and treatment consumption in control trials suggests that the disturbance of uncovering the stimulus chamber did not

affect oxygen consumption, so differences in consumption during other trials were due only to the contents of the stimulus chamber.

When a snake was visible, lizards reduced oxygen consumption by about half, which shows that the immediate effect (i.e., aerobic response) of visual contact with a predator was a significant reduction in aerobic energy use below the aerobic energy use of a calm lizard. If the lizard uses anaerobic respiration during this time, then future energy use may increase to process the resulting lactic acid. Reduction in oxygen intake could be due in part to reduction in breathing rate to minimize movements that could draw the predator's attention (Labra and Leonard, 1999). Lizards likely chose this strategy instead of attempting to flee because the snake was close enough that an attempt to flee would have increased the likelihood of detection. Lizards maintained immobility and reduced oxygen consumption during the 10 minutes when the snake was no longer visible, suggesting that they were not certain that the snake was no longer a threat.

Behavior was not quantified during the oxygen consumption trials, but I did note some trends. Lizards showed more variation in behavior during the prey trials than during predator or control trials: even though all lizards had been fed uniformly, they had varied interest in the crickets. Perhaps a longer fasting period (3 or 4 days) would make lizard reactions to prey stronger or more uniform. It is possible that some anoles did not feel comfortable enough in the environment (i.e. the flask in the cardboard box) to chase prey. Variation in behavioral response could be due to basking behavior prior to trials, as body temperature affects physiological processes useful in prey capture such as burst speed and endurance (Huey, 1982).

This experiment did not take anaerobic energy into account. If anoles use less oxygen due to making fewer movements and instead rely on anaerobic metabolism, they may build up lactic acid in their muscles that will require energy to process in the future and may limit their ability to make quick movements in escape. Future studies could examine the amount of lactic acid buildup in anoles' muscles when predators or prey are visible. Future studies could also compare the responses of female lizards to male lizards, as it is possible that females assign different importance to predators and prey, especially while gravid. A more excitable species of anole such as *A. sagrei* might show more dramatic changes in oxygen consumption.

Behavior-

Unlike the oxygen consumption trials, in which the anoles had limited auditory and olfactory information about the stimulus chamber since they were in an enclosed flask, behavioral trials involved limiting only sight when the stimulus chamber was covered. If the anoles were unaware of the contents of the stimulus chamber before the contents became visible, one would expect a significant difference in behavior based on whether the stimulus chamber was covered or uncovered. There was no noticeable difference. The lizards may have detected the crickets and snake by smell or sound when the stimulus chamber was covered. This information about the contents of the stimulus chamber could account for the lack of difference in behavior when the stimulus chamber was covered or uncovered, when there was a difference in behavior when the chamber contained prey or predator.

Anoles made the same number of body movements per minute and gaze changes per minute during prey trials and control trials. This is similar to observations made

during the oxygen consumption experiment, when anoles kept their oxygen consumption at baseline levels in both prey trials and control trials. Even though the behavioral trials took place in a more naturalistic setting (i.e. the lizard was not contained in a flask but free to move in its home cage), lizards did not respond behaviorally to the prey, just as they did not respond metabolically. Perhaps the lizards would have responded if they had been fasted for more than two days.

Predator trials elicited significantly fewer body movements per minute and changes of gaze per minute than prey and control trials. Body movements tended to place the lizard outside the snake's field of view (i.e. behind the snake's head) if the snake was not moving. If the snake was moving, the lizards tended to make fewer body movements. These results also support the results of the oxygen consumption trials: the immediate response of lizards seeing a predator was reduced movement and reduced oxygen consumption. These results do not take into account anaerobic respiration, which could require greater energy use in the lizard's near future due to the need to process lactic acid build-up. This could limit the lizard's ability to move quickly, so perhaps immobility is the least costly antipredator strategy only if it is used for a short enough time that a quick escape is still possible.

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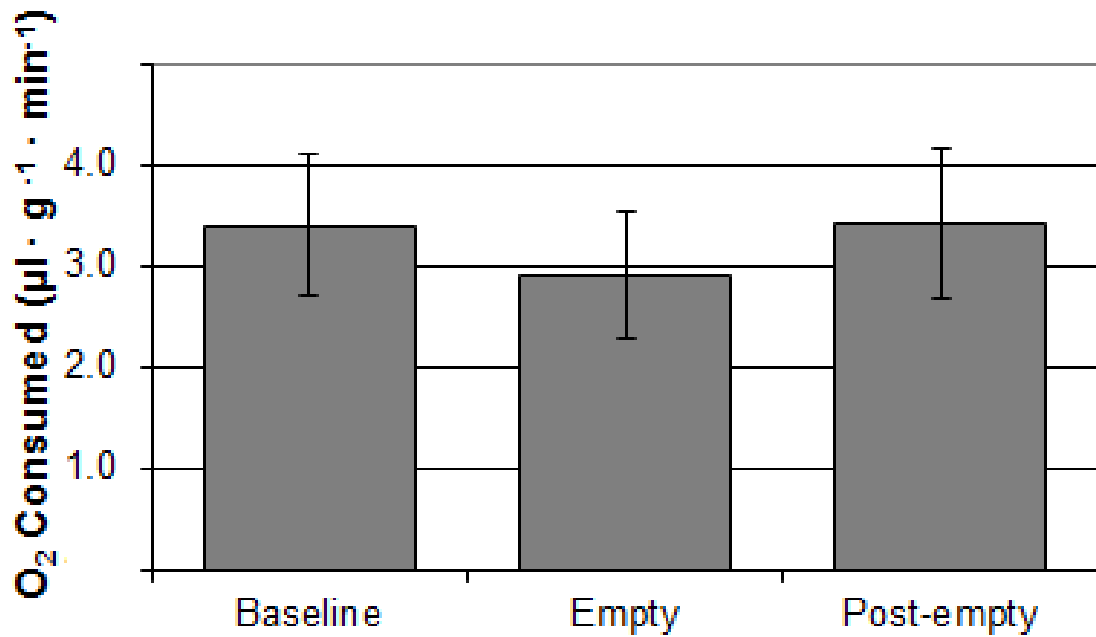


FIG 1 – Mean oxygen consumption of lizards measured in $\mu\text{l O}_2 \cdot \text{g}^{-1} \text{ lizard mass} \cdot \text{min}^{-1}$ \pm SE during control trials. Oxygen consumption was measured before the trial with the stimulus chamber hidden (“baseline”), while each anole could see an empty chamber (“empty”), and after the chamber was covered (“post-empty”). $n = 13$.

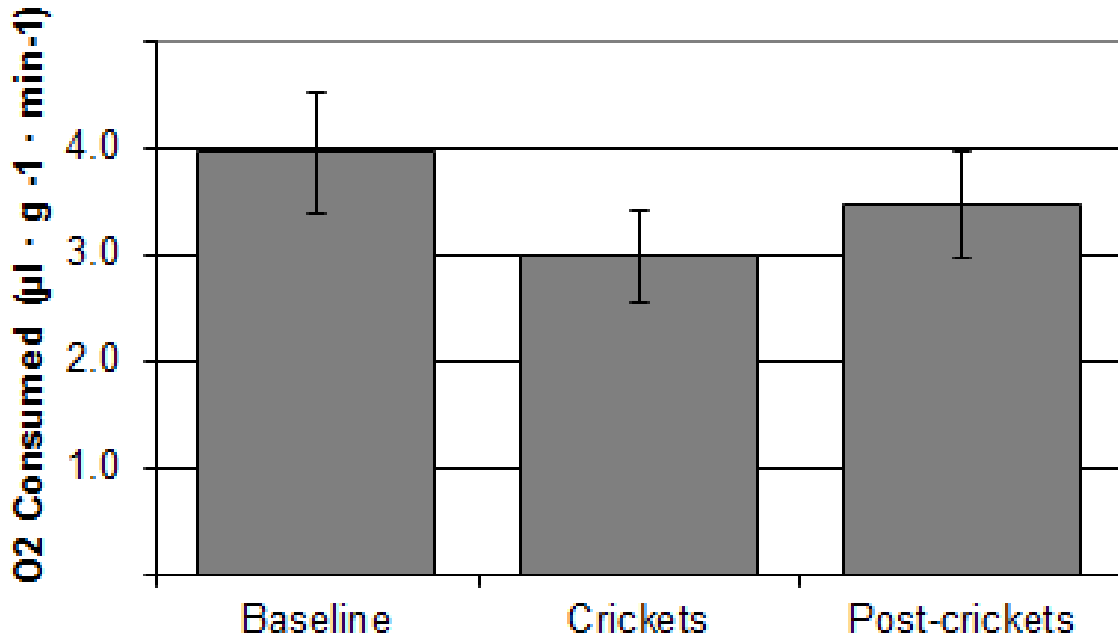


FIG 2 – Mean oxygen consumption measured in $\mu\text{l O}_2 \cdot \text{g}^{-1} \text{lizard mass} \cdot \text{min}^{-1} \pm \text{SE}$ during prey trials. Oxygen consumption was measured before the trial (“baseline”), while each anole could see a chamber containing insect prey (“crickets”), and after the chamber was covered (“post-crickets”). $n = 13$.

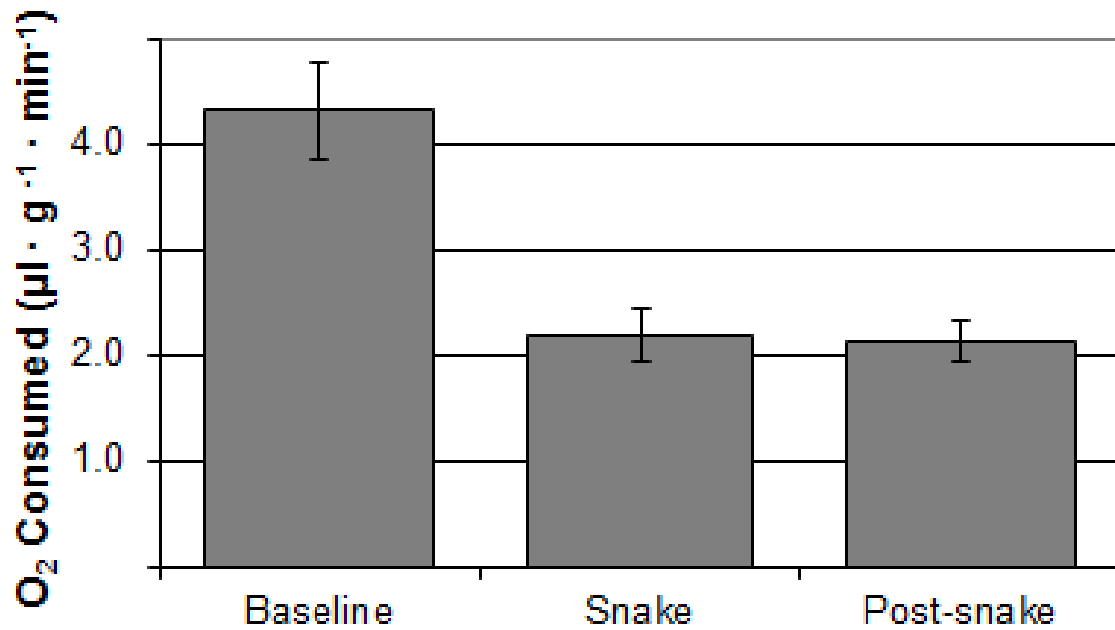


FIG 3 – Mean oxygen consumption measured in $\mu\text{l O}_2 \cdot \text{g}^{-1} \text{ lizard mass} \cdot \text{min}^{-1} \pm \text{SE}$ during predator trials. Oxygen consumption was measured before the trial (“baseline”), while each anole could see a chamber containing a predator (“snake”), and after the chamber was covered (“post-snake”). $n = 13$.

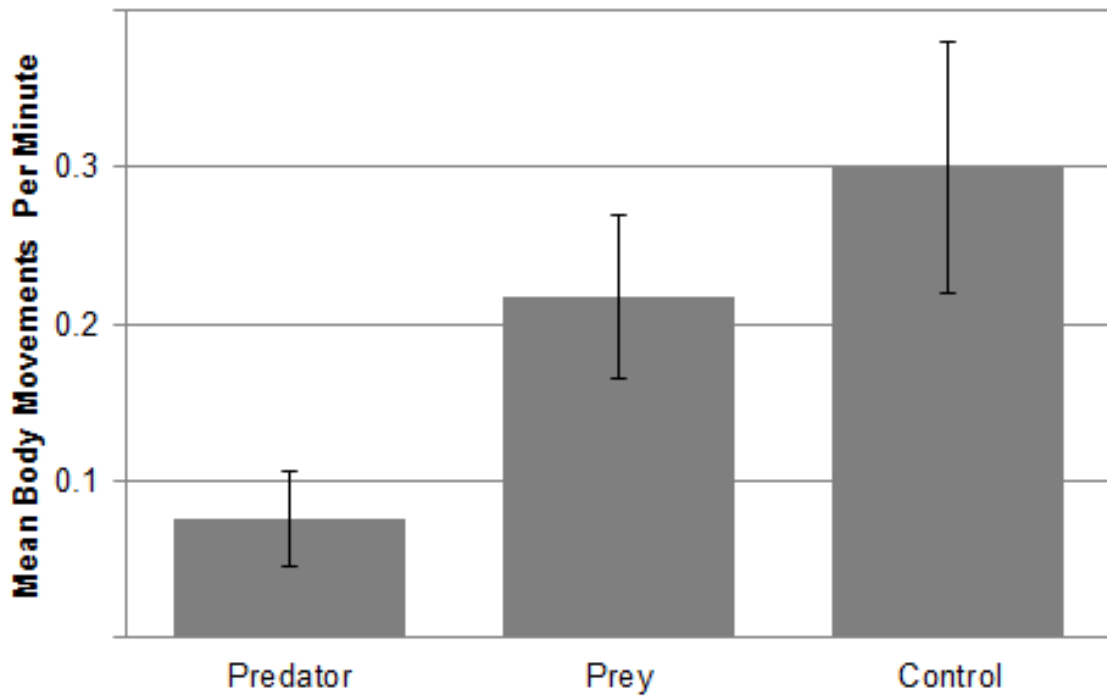


FIG 4 – Mean number of body movements per min. during the 10 minute interval when an anole could view a cornsnake (“predator”), insects (“prey”), or an empty chamber (“control”). Vertical bars are \pm SE. $n = 12$.

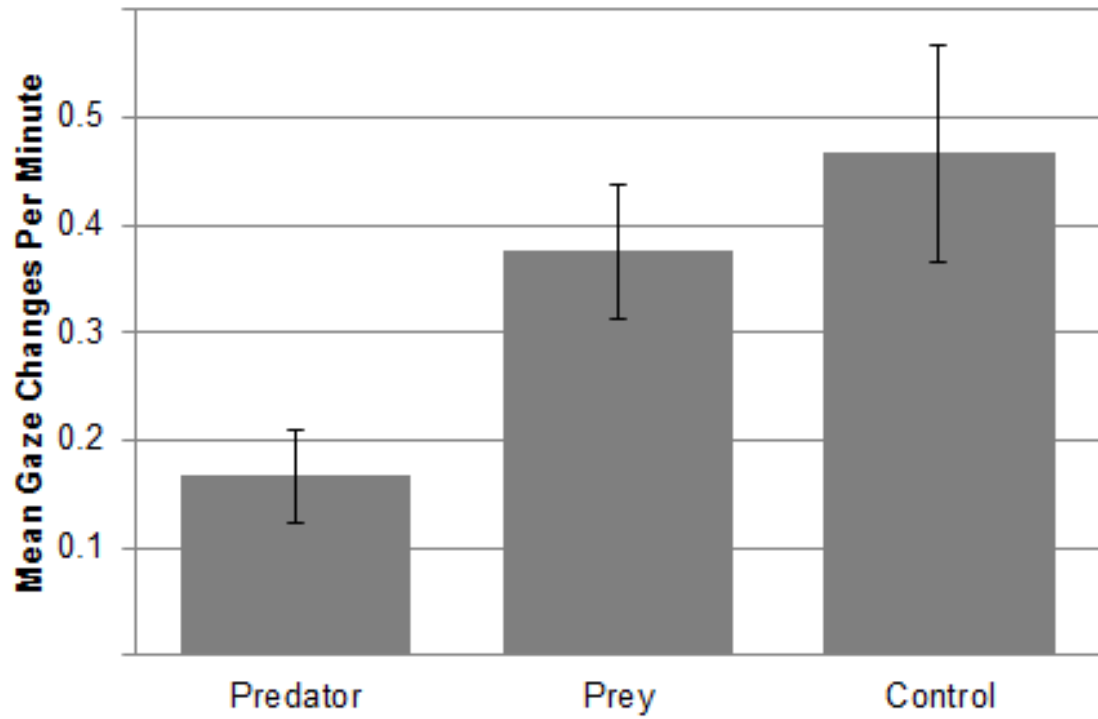


FIG 5 – Mean number of gaze changes per min. during the 10 minute interval when an anole could view a cornsnake (“predator”), insects (“prey”), or an empty chamber (“control”). Vertical bars are \pm SE. $n = 12$.

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Metabolic Response to Visual Contact with Predator and Prey
in the Green Anole (*Anolis carolinensis*)
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