

TEACHING TAXONOMY, THERMOREGULATORY BEHAVIOR, AND PHOTOPERIOD
USING LABORATORY OBSERVATIONS OF *ANOLIS CAROLINENSIS*

A Report of a Senior Study

by

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ABSTRACT

Meeting the state teaching standards for taxonomy, interactions of animals and the environment, and scientific methodology can be difficult for teachers, but the development of an interesting experiment can engage students and encourage learning in the classroom. Two experiments with *Anolis carolinensis* were developed to meet these standards in the high school classroom; these experiments were designed to examine the affects of varied photoperiod and temperature on light orientation, movement, body orientation, and coloration. Experiment 1 examined how seasonal-specific photoperiod with constant temperature affects the thermoregulatory behaviors of *Anolis carolinensis*, and experiment 2 examined the affects of seasonal-specific temperature with constant photoperiod. No significant differences in light orientation existed in varied photoperiod ($P = 0.077284$) or varied temperature ($P = 0.880178$). No significant differences in movement were exhibited in varied photoperiod ($P = 0.783655$), however significant difference was exhibited in varied temperature ($P < .005$). Tabulated body orientation showed that burial orientation with aggregation was only observed in the 5°C temperature condition. Coloration also changed significantly with varied temperature. This experiment provides a teacher the opportunity to use an experimental activity to reinforce difficult curriculum. With minor classroom-specific modifications, implementation of this experiment can be achieved in most high school biology classrooms.

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CHAPTER I

INTRODUCTION

Species interaction and response to environmental stimuli is a biological concept that has been heavily researched and observed, especially in animal and plant species. The effects of environmental stimuli have caused species to evolve different adaptations that promote survival. As an example, seasonal-specific associated photoperiod and thermoregulatory behaviors have allowed species to thrive in their environment and co-exist with other species. The thermoregulatory behavior of a species helps to determine a species' ability to adapt to various climates, ecological range, and habits. (See Table 1 for definitions of scientific terms such as thermoregulation.) The body temperature of a species can affect their ability to adapt to an environment, and associated photoperiods are important for species' seasonal acclimation and separating species' interactions.

Teaching species interaction to environmental stimuli can be difficult for middle school and high school teachers because students often know the basics of this scientific principle through previous interactions with the environment; however, linkage/integration of the background knowledge and the complex concepts or definitions may seem too difficult for some students. Student difficulty may arise because they have never associated these biological terms

and concepts with visual stimuli. Explanation of these abstract concepts through instruction is often not enough for students, so teachers must apply these concepts to tangible observations. It can also be difficult to teach these concepts because some students have the natural ability to comprehend material faster, whereas it may take repetitive explanation to relay the ideas of a concept to other students. It is a challenge to keep all students engaged with thought provoking material. Relating the concepts of this experiment to each student's real life experience and application can be difficult but is critical to student engagement and enthusiasm.

Table 1. A glossary of scientific terms associated with this exercise, which should be referred back to while reading through this document

Adaptation- the evolutionary process by which individuals and species become better suited to their environment
Aggregation- collection of more than one individual of a species in the same location
Associated Reproduction- formation of gametes and sex hormones is associated with mating and fertilization
Circadian Rhythm- a roughly-24-hour cycle in the biochemical, physiological or behavioral processes of living beings differentiated by distinct photoperiods
Continuous Reproduction- where gametes are formed continuously throughout the adults reproductive maturity
Dissociated Reproduction- species produce sperm or eggs at some time other than during the mating season and stores them until mating occurs
Diurnal- a species that is active during the light cycle of a photoperiod
Ectothermic- species where the source of internal body temperature is the external environment
Ethogram- a graphic representation of an animal's normal behaviors and interactions; used to quantify behavior
Eukaryote- organisms composed of cells containing a membrane-bounded nucleus and other membrane bound organelles
Photophase- the light period of a photoperiod cycle
Photoperiod- reoccurring cycle of light and dark periods
Scotophase- the dark period of a photoperiod cycle
Taxonomy- A systematic method of classifying plants and animals. Classification of organisms based on degrees of similarity purportedly representing evolutionary (phylogenetic) relatedness
Thermoregulation- a species' regulation of body temperature (termed ectothermic if the source of heat is from the environment and endothermic if the source of body heat is internal)
Thermoregulatory behavior- adaptive behaviors associated with a species' thermoregulation

Background Information

Depending on the geographical location of a school, students may be familiar with small lizards and/or other ectothermic species associated with the region. If students are aware of these types of animals, they can often identify them based on their common name. The teacher can, and should, often refer to a species by its common name so that students are aware of the species which is being discussed. These common names allow individuals to identify species based on their regional differences and can aid in engaging the students. The lizard species *Anolis carolinensis* is no exception, having multiple common names such as Green Anole, American Chameleon, and Carolina Anole; however, a drawback to this common name identification is that it is not universal. A teacher's repetitive referral to a species by its common name allows for student recognition and distinction, but this practice should not be used exclusively, especially within the confines of scientific writing. Taxonomic classification is a universally-accepted means of naming and classifying living things based on Latin nomenclature, and this identification method is critical to a proper understanding of Biology (Case 2008). Scientific identification of species is based on binomial nomenclature, where both genus and species are used to identify the organism. Complete taxonomic classification for *Anolis carolinensis* is shown in Table 2.

Table 2. Descriptive representation of classification for the taxonomy of *Anolis carolinensis*

Taxonomy	Classification	Description
Kingdom	Animalia	In general, multicellular Feed on other organisms, capable of locomotion, responsive to environment
Phylum	Chordata	Notochord at some stage, complete digestive system, endoskeleton usually present, segmented body, closed blood system
Subphylum	Vertebrata	Spinal column present, central nerve cord, ventral heart 2-4 chambers, paired kidneys, muscles attached to endoskeleton
Class	Reptilia	Eggs have amnion, fertilization occurs inside female, have epidermal scales made of protein
Order	Squamata	Movable quadrate bones, males have a hemipenis, viviparous, ovoviviparous, and oviparous species
Suborder	Iguania	Anoles, Chameleons, Iguanas
Family	Polychrotidae	Oviparous (produce eggs outside body), most arboreal
Genus	Anolis	Morphological changes associated with habitat
Species	carolinensis	Typically green but range to brown, 5-8 inches, pink dewlap, lay a single egg

Anolis carolinensis is a slim, medium sized lizard (12.7 to 20.3 cm in length as adults), with a pointed head and long tail which can be up to twice the length of the body. *Anolis carolinensis* is an adept climber and uses transverse lamellae on the bottoms of the toes and feet to gain traction. Transverse lamellae are thin structures with tiny grooves in the epidermis of the animal that allow it to grip small irregularities on a surface, and are shed just as any other part of a lizard's epidermis. *Anolis carolinensis* is primarily an insectivorous feeder but also preys on arthropods such as small spiders in the wild. This species is a diurnal feeder. Anoles stalk their prey and must see their prey move to detect it. This species is not threatened and is most commonly used for pet trade by humans (Hutchins 2003). The distribution of the green anole in the southeastern United States ranges from upper North Carolina to Key West Florida and from southeast Oklahoma to central Texas. *Anolis carolinensis* has been introduced in the Hawaiian Islands and is also found on the island of Cuba and many other Caribbean islands (Conant and Collins 1991). This species prefers a habitat consisting of a moist climate, vegetated areas, and

many climbing surfaces, which explains the geographic distribution. The habitat and climate to which this species associates also affects this species reproductive biology.

There are three general reproductive cycles in reptiles and amphibians, and the cycles are associated, dissociated, and continuous. Because of the predictable environment in which this species lives, *Anolis carolinensis* has developed an associated reproductive cycle. In this cycle, the maturation and shedding of gametes and the secretion of sex steroid hormone are associated with mating and fertilization, and spermatogenesis and oogenesis are initiated very close together (Pough et al. 1998). In *Anolis carolinensis*, the growth of the gonads occurs in the spring, mating and egg production occur over the summer, and gonads regress in both male and female in the fall (Pough et al. 1998). The photoperiod also has an effect on the initiation and sustention of spermatogenesis in the species. In optimal environmental conditions, Anoles produce more than one clutch per reproductive season. A female *Anolis carolinensis* produces single eggs every seven to fourteen days, and the development of the egg determines its receptivity to courtship (Pough et al. 1998). The behaviors of *Anolis carolinensis* during courtship can vary between different populations, but some behaviors are consistent with populations of this species.

Anolis carolinensis is highly territorial, especially during the mating season, and their interactions with females help determine some of their behaviors. This species uses extensive visual communication to establish and defend territory as well as find and attract mates. Male anoles are typically larger than their female counterparts of similar age, and males have a throat fan, known as a dewlap, which range in color from pink to red (Hutchins 2003). Male *Anolis carolinensis* utilize headbob patterns, extension and retraction of the dewlap, dewlap coloration, and intricacy of display to express dominance and find a mate. The dewlap color is also important to species identification and species recognition. The vigor of the males display seems

to be the most important component for the attraction of females (Zug et al. 2001). Males may also exhort their dominance over other males by erecting the dorsal skin into a crest and/or engaging in stereotypical posturing to enlarge their body image (Hutchins 2003).

Color change is a characteristic of this species and is associated with particular behaviors. Anoles have the ability to change its usual green coloration to one of brown or gray. Color change is presumably associated with temperature, stress, and various other environmental and behavioral factors (Porter 1972). Basking is another behavior of this species, and is critical to *Anolis carolinensis* survival because it is ectothermic. Basking occurs when an animal sits still on a surface and absorbs the heat from sunlight, and this technique is used to raise body temperature and prepare the lizard for the day's activities (Hutchins 2003). This species is arboreal, and they most often climb to the area which they have designated for basking. During winter, northern populations of *Anolis carolinensis* may spend up to 92% of their emerged time basking in direct sunlight to regulate body temperature (Jenssen et al. 1996).

Thermoregulation

Behavioral thermoregulation is critical in ectothermic species because of an ectoderm's reliance on the environment to provide the heat necessary for metabolism, and many of these behaviors are dependent on species characteristics such as circadian rhythms, locomotor activities, basking, aggregation, and alterations in posture and color (Hutchins 2003). All ectotherms have optimal body temperatures within their species-specific preferred range, and for most lizard species this is between 35°C and 42°C. However, the mean body temperature for a winter-aggregated population of *Anolis carolinensis* at 33.5° north Latitude was 22.7°C, while the air temperature was 2.4°C lower (Jenssen et al. 1996). The significant difference in body temperature and air temperature of this specific population of *Anolis carolinensis* represents the

species use of thermoregulatory behaviors to raise body temperature. *Anolis carolinensis* uses each of these thermoregulatory characteristics to allow it to inhabit cooler climates without hibernating during a seasonal winter cycle (Bishop and Echternacht 2004). *Anolis carolinensis* is a species which often aggregates during winter seasonal cycles. The cooler climates associated with this Anolis population are characterized by longer and more severe seasonal winter cycles, but these behavioral adaptations allow this species to survive. The thermoregulatory behaviors associated with a particular species are affected by the species' daily rhythms.

Among reptiles, this daily rhythm of behavioral thermoregulation was first identified to be a circadian rhythm in the iguanid lizard, *Sceloporus occidentalis* (Cowgell and Underwood 1979). The circadian rhythm of a lizard is either diurnal or nocturnal, and *Anolis carolinensis* is a diurnal species (Porter 1972). The affiliation of a species with its particular photoperiod rhythm affects their thermoregulation by varying their specific preferred body temperature range and required behaviors necessary to maintain this body temperature. The mean preferred body temperature of *Anolis carolinensis* has two "preferred states," the photophase and scotophase state, and higher body temperatures are selected during the photophase than during the scotophase because of the species' diurnal activity (Gans and Crews 1992). Due to *Anolis carolinensis*' extensive ecological range, the northern most latitudinal populations of this species can have different thermoregulatory adaptations than the southern populations. These adaptations are what allow these northern populations to survive.

Photoperiod

Photoperiod affects behavioral thermoregulation in lizard species and is linked to the species' circadian rhythm. The daily rhythm for diurnal lizard species is based on its selection of warmer temperatures during the photophase and cooler temperatures during the scotophase of a

light : dark (LD) cycle (Regal 1967). Photoperiods based on a 24 hour light-dark cycle can be altered to test the effects of photoperiod on internal circadian clocks and characteristics of a species. A regions associated photoperiod may alter the times at which behaviors are performed or may alter behaviors entirely. Based on the seasonal temperatures and climate, the daily rhythms of lizards may change to accommodate their thermoregulatory needs. Circadian rhythms are critical because all eukaryotic species must coordinate their internal circadian rhythms to the established environment conditions in order to maximize fitness, and circadian rhythms differ among species (Emerson et al. 2008). It is still not known if the daily rhythm of behavioral thermoregulation is controlled by the circadian system in all reptile families or whether the expression of this circadian rhythm is subject to seasonal variation (Ellis et al. 2006).

The winter temperatures in temperate climates cause lizards to adopt a pattern of acclimation, or dormancy, and in the northern populations of *Anolis carolinensis*, the species often aggregates for thermoregulatory purposes and uses basking patterns to raise body temperatures into the activity range (Jenssen et al. 1996; Bishop and Echternacht 2004). *Anolis carolinensis* exposes its body surface to sunlight to raise its core body temperature and upon achieving this preferred temperature, uses locomotor activities to move its body between light and shade (Porter 1972). Northern populations of *Anolis carolinensis* use crevices in rock faces and burrows to maintain body temperature, and on typical winter days, lizards do not emerge from the crevices before the sun illuminated their crevice; on overcast winter days, lizards rarely emerg (Bishop and Echternacht 2004). *Anolis carolinensis* and other ectothermic species also use changes in body position and color variation to affect thermoregulation. Observations on a northern population of *Anolis carolinensis* showed that the lizards oriented their bodies laterally within a crevice opening and exposed an entire side to the sun before emerging completely from

the crevice (Bishop and Echternacht 2004). This behavioral pattern helps to increase the body temperature of the lizard and protected it from predators. This use of crevices can be used in various climates. Once a lizard can no longer maintain a safe body temperature, it ceases activity and retreats to a burrow so that it can cool or warm its body temperature (Pough et al. 1998).

Educational Standards and Experimental Purpose

Both national and state teaching standards require knowledge of taxonomy, interactions of animals and the environment, and scientific methodology. For example, the *National Science Education Standards* require that students, grades 9-12, have the “Abilities necessary to do and understand scientific inquiry” as well as understand that “Organisms have behavioral responses to internal changes and to external stimuli” (NSES 2009). While these standards are broad and hard to integrate into singular lectures, the concepts of experimental design and thermoregulatory behavior are good representatives of scientific inquiry and behavior responses to internal and external stimuli. These national education standards for behavioral responses are further refined in specific state curriculum standards. The *Tennessee Science Education Standards* for Biology I students, grades 9-12, require that, “students investigate the interactions of organisms with their environment through different nutritional relationships, population dynamics, and patterns of behavior” and that “students investigate the diversity among organisms by analyzing classification systems, exploring diverse environments, and comparing life cycles” (TDOE 2009). These national and state education standards help to provide a basis for student understanding of species diversity and interaction within the context of Biology.

The goal of this study is to design effective learning strategies to teach taxonomic classification and behavioral responses to external stimuli. To meet this goal two experiments have been designed. The purpose of the first experiment is for students to design two identical

artificial habitats, one representing a control and the other representing a manipulated environmental variable. Also in this first experiment, the students will observe how the species' behaviors differ when photoperiod is altered in its environment. The purpose of experiment two is for the students to observe the effect of temperature on species' behavior. The ability to differentiate between temperature and photoperiod also allows the students to explore how species are affected by different environments, and from this, they can compare how behaviors and life cycles are similar and/or different based on the environmental characteristics.

These experiments allow students to use scientific methodology to observe biological interaction and encourage the application of scientific inquiry through observation. These experiments allow students to be active participants in creating science, instead of just passively learning the material through a textbook and provide a way for visual and kinesthetic learners to connect to these more difficult biological concepts. After completing these laboratory observations, students will be able to: (1) classify animal species by their taxonomic groupings, (2) paraphrase the importance of taxonomic classification in their own words, (3) explain thermoregulation, (4) judge what would happen to an ectothermic species if it was not allowed to self thermoregulate, (5) describe a photoperiod, (6) collect and analyze data, and (7) make conclusions based on data collected during observations. This experiment also incorporates discussion of other scientific concepts: formulation of a hypothesis, experimental design, analyzing results to support a hypothesis, and communicating results scientifically.

CHAPTER II

MATERIALS AND METHODS

Two experiments were designed to test the affects of temperature and photoperiod on the thermoregulatory behaviors of the exothermic poikilotherm species, *Anolis carolinensis*. *Anolis carolinensis* (n=12) were obtained from Ward's Natural Science catalog cat number 87 W8135, in packs of 3 for \$16.75.

Experiment 1: Constant Temperature, Variable Photoperiod

Experiment one was designed to examine how seasonal-specific photoperiod affects the thermoregulatory behaviors of *Anolis carolinensis*. Based on the identification of characteristics associated with the lizard species *Anolis carolinensis*, the experimental design of two identical terrarium environments that met the needs associated with this species were developed.

Step 1: Tank set-up and Animal Introduction:

Two 10-gallon terrariums, equal amounts of soil or bedding for each tank, rocks of similar size and shape to place in the terrarium, and similar artificial foliage were needed for this experiment (see Figure 1). A 250 watt heat lamp was positioned on the right side of each tank,

about 25 cm above and to the right of the tank. The tank environments were identical to one another so that behaviors were linked directly to temperature and photoperiod and not other variables. The exterior of each tank was measured and divided into three equal sections, length wise and labeled right, middle, and left. Lizards were randomly divided into two different populations, each consisting of six lizards. A 2:4 male to female ratio was kept in each experimental population. Lizards were numbered from one to six in each population so that their specific movements could be documented (The numbering of each specimen was based off of physical attitudes). One population was established as the control and the other as the variable population. The control population remained constant for temperature and photoperiod throughout the length of the experiment, while temperature and photoperiod were altered separately in the variable population.



Figure 1: Photographic representation of the terrarium and other associated set-up materials

Step 2: Climate Acclimation:

Before beginning the experiment, both populations of lizards were given two days to acclimate to the established control for photoperiod and temperature. The established control photoperiod was a 14L:10D cycle, and the established control temperature was 23°C (the average summer temperature in eastern Tennessee). After the two-day acclimation period the photoperiod of the variable population was changed to a 10L:14D photoperiod cycle.

Step 3: Data collection:

The designated photoperiods for this experiment were based on summer and winter seasons. The established control photoperiod cycle 14L:10D represented the seasonal summer photoperiod, and the winter photoperiod cycle was represented by a 10L:14D cycle. Before beginning observations, a table (called an ethogram) was formatted to accurately differentiate each lizard's characteristics and date of observation (see Table 3). To begin observations, the location of the each lizard in the terrarium (front, middle, or back in relation to the light source) and its body orientation to the light source was documented within the table. Lizards in the front were exposed to 950 LUX, while those in the middle and back were exposed to 800 and 500 LUX, respectively (as measured by a Vernier light intensity probe). The behavior of the each lizard (still or active) was observed and recorded. The lizard's body orientation, movement, position in tank, and color were recorded as an average for the 20 minute observation period. Both populations were subjected to the 20 minute observation. These observations were collected for five days on an experimental population of six lizards, for a total of 30 observations per experimental population.

Table 3. Representative ethogram used for observations of body orientation, movement, color, and tank orientation in experimental populations of *Anolis carolinensis*

		Population		
		Oriented Tank Section Illumination (Lux)		
		950	800	500
Body Orientation	Lateral			
	Perpendicular			
	G Lateral			
	G Perpendicular			
	B Lateral			
	B Perpendicular			
Movement	Frozen			
	Chill			
	Sloth			
	Rabbit			
Color	Green			
	Brown			
	Combo			

Body Orientation

Lateral Basking - LB - body is positioned laterally to the lamp (head or tail facing the lamp)

Perpendicular Basking - PB - body is positioned perpendicular to the lamp (left or right flank facing the lamp)

Glass Lateral Basking - GLB - clinging to the glass with head or tail facing the lamp

Glass Perpendicular Basking - GPB - clinging to the glass with flank facing the lamp

Burial Lateral- BL- body is submerged in the wood chip layer with head or tail facing the lamp

Burial Perpendicular- BP- body is submerged in the wood chip layer with flank facing the lamp

Movement

Frozen - F – no movement both eyes closed

Chill - C – no movement one or both eyes open

Sloth - S - slow movements (walking, slight head or body movement, ect.)

Rabbit - R – quick/fast movements (running, jumping, head bobbing, ect.)

Color

Combo - C - no one color is more prevalent than another

Step 4: Data analysis and Presentation:

Bar charts with standard error bars were created to present recorded observations on each specimen’s tank orientation and movement within the enclosure. The bar chart for light orientation was based on the total number of observations for each luminous region during

photoperiod experimentation. The bar chart for movement was based on a movement index of 1, with 1 representing all specimens at highest activity and 0 representing all specimens with no activity. Each movement was designated a number from 0-3 with 0 being frozen and rabbit being 3, and the daily total movement for each population was divided by the highest movement total possible to determine the daily movement index. The average daily movement index was totaled from the five day experimental period. A table was used to depict the number of observations recorded for each body orientation during experiments for photoperiod. Pie charts were used to represent the percent coloration of the two Anole populations during photoperiod experimentation.

Experiment 2: Constant photoperiod, variable temperature

Step 1: Set up and Climate Acclimation:

The same experimental setup used for experiment one was used for experiment two. After five days of photoperiod observation, the variable tank was returned back to the established control photoperiod cycle of 14L:10D. The lizards in the tank were allowed to acclimate to the control photoperiod for two days. After the lizards acclimated, the temperature of the tank was lowered to examine the affect of temperature on thermoregulatory behavior. The temperature of the variable group's enclosure was lowered to average winter temperature for East Tennessee 41°F or 5°C.

Step 2: Data collection:

Another table was formatted to record the observations associated with temperature. As in experiment one, the location of the each lizard in the terrarium (front, middle, or back in relation to the heat source) and its body orientation to the light source was documented within

the table. The behavior of the each lizard (still or active) was observed and recorded. The lizard's body orientation, movement, position in tank, and color were recorded as an average for the 20 minute observation period. Both populations were subjected to the 20 minute observation. These observations were collected for five days on an experimental population of six lizards, for a total of 30 observations per experimental population.

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Bar charts with standard error bars were created to present recorded observations on each specimen's tank orientation and movement within the enclosure. The bar chart for light orientation was based on the total number of observations for each luminous region during temperature experimentation. The bar chart for movement was based on a movement index of 1, with 1 representing all specimens at highest activity and 0 representing all specimens with no activity. Each movement was designated a number from 0-3 with 0 being frozen and rabbit being 3, and the daily total movement for each population was divided by the highest movement total possible to determine the daily movement index. The average daily movement index was totaled from the five day experimental period. A table was used to depict the number of observations recorded for each body orientation during experiments for photoperiod. Pie charts were used to represent the percent coloration of the two Anole populations during temperature experimentation.

CHAPTER III

RESULTS

A total of 120 observations for tank orientation, movement, body orientation, and color were recorded for each population of *Anolis carolinensis* during established experimental periods for photoperiod and temperature. Each individual in a population was designated a number, and the individual's actions were recorded. Data recorded represents the average location, position, or behavior of each animal during a 20 minute observation period which began 1 hour after experimental "sunrise".

Anolis orientation to the light source based on experimental conditions is represented by Figure 2. Each experimental condition was observed ($n = 30$) and for light orientation the tank was sectioned into 950, 800, and 500 LUX sections. For the 14L:10D photoperiod, mean Anole location was 760 LUX, whereas for 10L:14D photoperiod it was 835 LUX ($P = 0.077284$). For the 23°C temperature, mean Anole location was 840 LUX and for 5°C was 845 LUX ($P = 0.880178$).

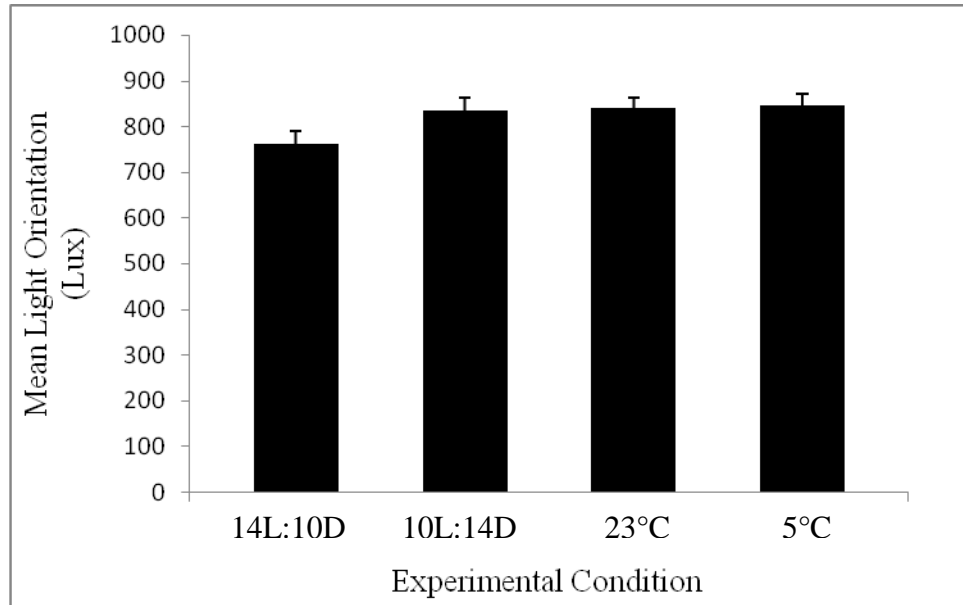


Figure 2: Mean light orientation (+SE) of *Anolis* in an illuminated tank section during experiments for photoperiod and temperature

The effect of experimental conditions on specimen movement was calculated by a movement index. Figure 3 represents each experimental conditions mean movement index from five days of index observations. The mean movement index for the 14L:10D photoperiod population was 0.476 (+SE) of 0.0932, and the mean index for the 10L:14D population was 0.446 (+SE) of 0.0498 ($P = 0.783655$). The mean movement index for the 23°C population was 0.442 (+SE) of 0.0466, and the mean index for the 5°C population was 0.024 (+SE) 0.0147 ($P < .005$).

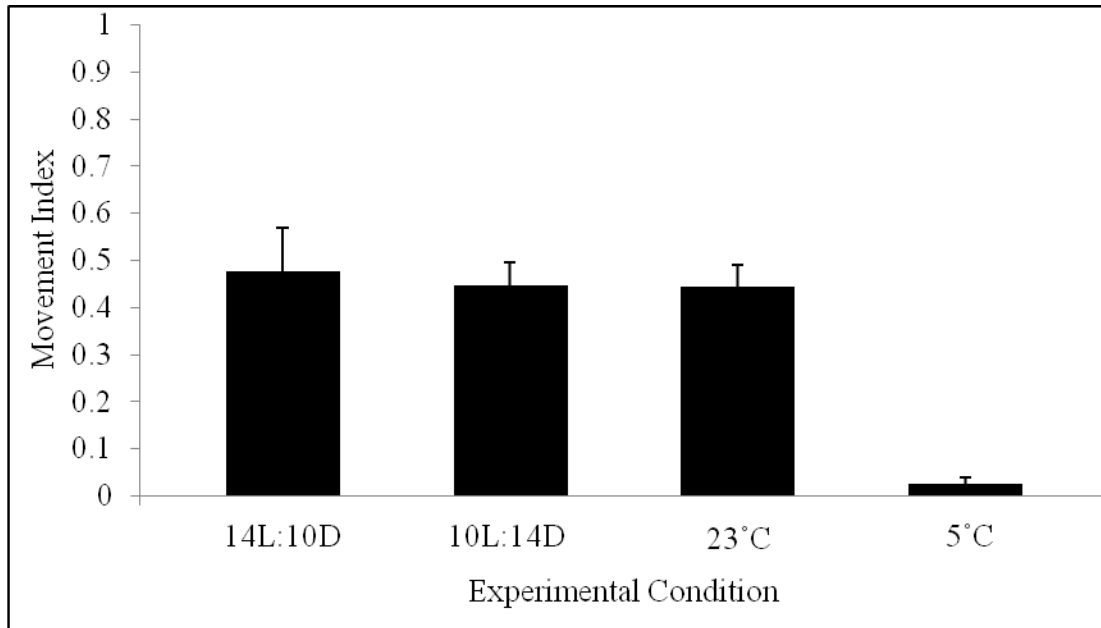


Figure 3. Measure of Anolis movement (+SE) in established experimental conditions for photoperiod and temperature, as measured by a movement index

Anolis body orientation varied based on experimental condition. Each experimental conditions total orientation reflects the impact of light on the population (Table 4). Lateral body positioning was observed in each population and represented the majority of observations for each experimental condition except for 5°C. Perpendicular orientation was observed the second greatest number of times in all of the conditions except for the 10L:14D photoperiod condition, and even in this condition it was observed only one less time than second most highly observed orientation. Glass orientation only represented a significant proportion of observations in the 10L:14D photoperiod condition, and burial orientation was only observed in the 5°C temperature condition.

Table 4: Number of observations recorded for each body orientation during experiments for photoperiod and temperature

	Lateral	Perpendicular	Glass Lateral	Glass Perpendicular	Buried Lateral	Buried Perpendicular
14L:10D	17	8	4	1	0	0
10L:14D	13	8	9	0	0	0
23°C	18	9	1	2	0	0
5°C	2	7	2	0	13	6

Anolis coloration varied based on experimental conditions for photoperiod and temperature. Percent coloration of *Anolis* populations observed during experimental conditions is represented by Figure 4. Coloration was similar in the control population which maintained a 14L:10D photoperiod and 23°C temperature during both experimental periods. The 10L:14D photoperiod increased percentages of brown and combo observations when compared to the 14L:10D photoperiod. Varied coloration in the 5°C condition was not present as all specimens were brown for each color observation over the five day observation period.

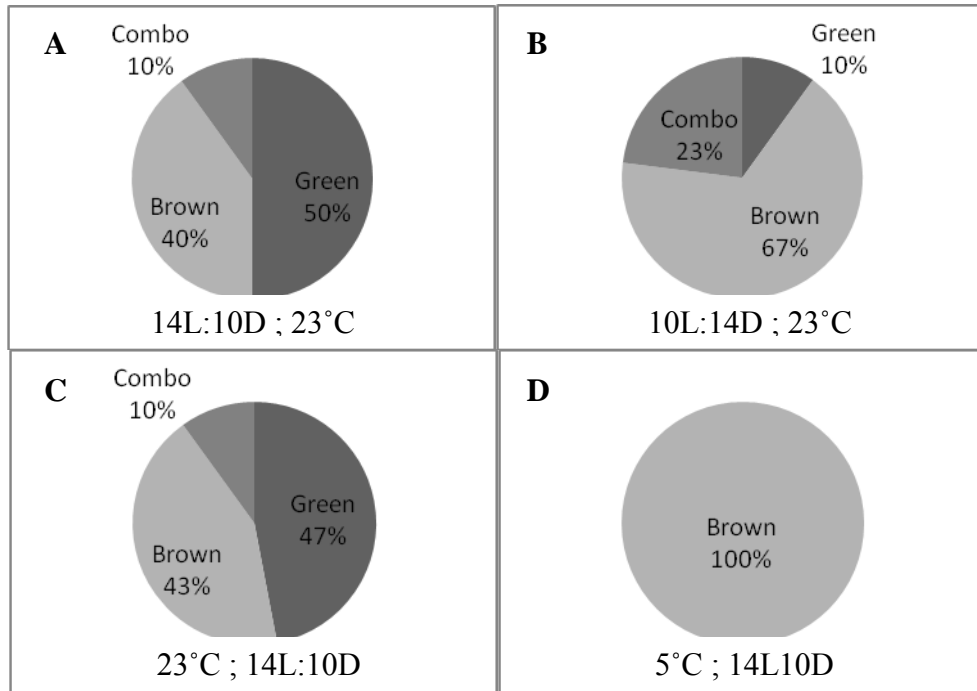


Figure 4. Percent coloration of lizard populations during experiments for varied photoperiod and temperature. Data from experiment one with (A) Summer conditions and (B) Summer temperature, Winter Photoperiod, as well as experiment two with (C) Summer Conditions and (D) Winter temperature, Summer photoperiod.

CHAPTER 4

DISCUSSION

The combined results for tank orientation, movement, body orientation, and color indicate that *A. carolinensis* body orientation to a light source is not solely depended on photoperiod or temperature, but movement and coloration were dependent on temperature and not photoperiod. Figure 2 p-values indicate that neither a varied photoperiod with a constant temperature nor a varied temperature with a constant photoperiod resulted in significant difference in orientation to the light. These data were not consistent with previous data for wild populations of Anolis. Bishop and Echternacht (2004) identified strong light orientation behaviors in wild populations of Northern Anoles, and lizard emergence was based on temperature and sun illumination. In varied photoperiod conditions with similar temperatures *A. carolinensis* did not exhibit significant difference in movement, but *A. carolinensis* did exhibit significant movement differences when subjected to similar photoperiods and varied temperatures (Figure 3). The cooler temperature caused a significant decrease in Anole movement as expected based on the poikilothermic regulation of a lizard species. Variation in locomotor activity during separate photoperiod conditions has been observed in some lizard species, but circadian rhythm effects on *A. carolinensis* movement have yet to be determined (Ellis et al. 2006).

Lizard body orientation was similar in each of the experimental conditions except for Winter temperature, Summer photoperiod. This was the only condition in which the lizards displayed burial behavior and grouped together while representing “frozen” movement behavior (Table 4). Previous experimentation with Northern populations of Anoles has indentified similar behavioral characteristics of aggregation and underground housing, and while there were thermal benefits for underground housing, there did not seem to be any thermal benefits of aggregation (Bishop 2004). Aggregations occur when access to key resources such as food, basking sites, oviposition sites, and mates is restricted because of their limited availability (Graves and Duvall 1995). Studies done on tadpoles suggest that aggregations and close physical proximity may benefit poikilotherms by facilitating feeding or thermoregulation (Smith and Jennings 2004). Future research into the physical conditions, social attraction (or avoidance), chance, and other factors affecting aggregation of lizard species, specifically *Anolis*, should be undertaken to determine the forces driving this behavior.

A. carolinensis coloration was varied between photoperiod and temperature conditions, but temperature had the greatest affect on lizard coloration. Winter photoperiods induced brown and combo colorations, whereas summer photoperiods resulted in more green coloration. Similar coloration was observed during the two tests for the “summer condition” (50% of observations in experiment 1 and 47% in experiment 2); however, winter temperature and summer photoperiod resulted in solely brown coloration in the lizard population (see Figure 4). Temperatures have been found to induce color change in reptiles, especially lizards, where cooler temperatures induced a darker coloration phase and warmer temperatures induced a lighter coloration phase (Porter 1972). The darker coloration observed in the winter temperature condition is supported by Porter’s explanation of coloration in lizard thermoregulation. Significant winter color change

could be due to changes in winter vegetation coloration or increasing thermoregulation by darker colors and thereby increasing amount of heat absorption from the sun during basking.

Classroom Integration

This experiment is an activity that can be used in a high school Biology classroom to teach an understanding of scientific methodology, taxonomic groupings, thermoregulation, and thermoregulatory behavior of a poikilothermic species in relation to environmental variation.

An experiment of this type can be incorporated into most all high school biology classrooms, but it may be difficult to accurately decrease temperature to winter conditions and maintain summer photoperiod conditions in the high school classroom because of the need for a glass door refrigeration unit. Variations to the environmental conditions can be implemented so long as significant variation exists for photoperiod and temperature. Variations to this experiment could include a base temperature and an increased temperature, altered photoperiod, and habitat condition. Such subtle alterations to varying environmental factors would likely need to be implemented due available funds and resources of a particular school or school district, and school officials should be contacted before any experimentation with animals in the classroom begins.

This experiment provides a teacher the opportunity to supplement an activity into teaching while increasing tangible curriculum involvement by each student. This activity also allows a teacher to breakup lectures into smaller segments, which is critical to maintain student attention. This activity can increase students' understanding of scientific methodology, content knowledge standards, and scientific inquiry (see Table 5). "Student interaction with organisms is one of the most effective methods of achieving many of the goals outlined in the *National Science Education Standards*" (NSTA 2008). Allowing students to observe and work with

animals in the classroom can increase student involvement and interest in subject matter. This activity can encourage students to become involved in future scientific study and endeavors by providing an interactive and engaging environment for science. The need for scientifically minded students is continually increasing in today's society, and all available opportunities to increase the educational experience for these students should be implemented.

Table 5. Addressing academic standards through the use of an experimental exercise

TN Curriculum Standards	Addressing the Standards	Assessment of Standard
Inquiry 2 Design and conduct scientific investigations to explore new phenomena, verify previous results, test how well a theory predicts, and compare opposing theories.	The students will be able to conduct an experiment testing the affects of variable photoperiod and temperature on <i>Anolis carolinensis</i>	Participation in the experimental process
Inquiry 3 Use appropriate tools and technology to collect precise and accurate data.	The students will be able to use light sensors, ethograms, and analyzing software to collect and form data.	Collection of ethograms to be graded for completion
Inquiry 4 Apply qualitative and quantitative measures to analyze data and draw conclusions that are free of bias	The students will be able to write several paragraphs on their experimental findings	Paragraphs will be typed collected for grading.
Inquiry 5 Compare experimental evidence and conclusions with those drawn by others about the same testable question.	The students will be able to present their experimental data to the class in a creative form.	Presentation of diagrams and figures in a creative form to be graded
Inquiry 6 Communicate and defend scientific findings.	The students will be able to discuss their experimental findings.	Participation in class discussion of results
Biodiversity and Change 5.1 Associate structural, functional, and behavioral adaptations with the ability of organisms to survive under various environmental conditions.	The students will be able to explain why the animals might have exhibited certain behavioral characteristics depending on experimental condition.	Paragraphs will be typed collected for grading.

APPENDIX

MARYVILLE COLLEGE
Institutional Animal Care & Use Committee (IACUC)
Student Animal Research Form

Student Name: Kyle Wayne Chewning

Student Email Address: kyle.chewning@my.maryvillecollege.edu

Date: 12 April 2009

Senior Study Advisor: Dr. Drew Crain

Species to be used: *Anolis carolinensis*

Age of animals: Mature Adults

Number of animals in study: 12

Duration of study: 3 weeks

Location of animals during the study (building and room): Sutton Science Center
Room- 103

List personnel to call if problems with animals develop:

Name	Daytime Phone	Nighttime Phone	Emergency No.
Kyle Chewning	(770) 654-9743	(770) 654-9743	
Dr. Drew Crain	(865) 981-8238		

Husbandry Requirements: Is anything other than routine care and equipment required?
YES _____ No __ If "YES", please list below.

What will happen to the animals at the end of the study? If euthanasia is required, state the methods.

At the end of the study, the animals will be given away as pets to individuals who have the ability and facilities to care for this particular lizard species.

(Do not write below line: For MC IACUC Use)

Maryville College IACUC Approval Number: _____

Date Approved: _____

Signed: _____

Is it likely that pain/discomfort will be experienced by animals in this protocol?

YES___ NO___If "YES", describe:

Pain or Distress Category: __ (See listing of Pain or Distress Categories below)

For categories C, D, or E, USDA regulations require that the investigator consider alternative procedures. Please provide a narrative (for instance the end of Chapter 1) describing the methods and sources used to determine that alternatives are not available. If a computer assisted literature search was conducted, provide the names of the database(s) and date(s) of the search.

Pain or Distress Categories

A. ACUTE STUDIES

Studies performed under anesthesia from which the animals are not permitted to regain consciousness, or performed on excised animal tissues collected under anesthesia or following euthanasia.

B. PAIN OR DISTRESS - NONE OR MINOR

Chronic studies that DO NOT involve survival surgery, induction of painful or stressful disease conditions, or pain or distress in excess of that associated with routine injections or blood collection. Included are induction or transplantation of tumors in animals (so long as the tumors do not cause pain and the animals are terminated prior to becoming seriously ill), administration of mildly toxic substances or drugs that cause no significant disease or distress, and antibody production as long as significant disease does not result and antigen booster doses do not include Complete Freund's Adjuvant (CFA).

C. PAINFUL PROCEDURES WITH ANESTHESIA/ANALGESIA

- a. Survival surgical procedures.
- b. Painful or potentially painful non-surgical procedures; e.g. bone marrow taps, injections into particularly sensitive areas such as foot pads, cardiac punctures, or traumatic procedures such as burns (burns may be category D, depending on severity).

D. MODERATE DISTRESS OR PAIN GENERALLY WITHOUT ANESTHESIA/ ANALGESIA/ TRANQUILIZERS

Induction of moderately distressful or painful disease conditions (examples: arthritis, administration of toxic chemicals, infectious challenges, immunosuppression resulting in infectious disease, peritonitis, severe inflammation, especially of weight bearing surfaces or resulting in external sores), whole body irradiation, stress models, septic shock, hypotensive shock, moderate painful stimuli (examples: low level electrical shock or heat), survival surgical procedures that have the potential to result in long term distressful illness (organ transplants, for example), induction of cardiac ischemia, booster immunizations with CFA, tumor induction or animal cultures that cause significant distress or pain, sight deprivation, restraint for periods longer than 12 hours.

E. INTENSE SUSTAINED OR REPEATED PAIN WITHOUT ANESTHESIA/ANALGESIA

Direct stimulation of CNS pain tracts, nociceptor stimulation by physical or chemical means that causes severe pain (e.g., corneal abrasions), or any category C (see above) procedure if performed without chemical relief of pain.

Investigator Assurance

Check all boxes that apply.

- The information provided in this protocol form accurately reflects the intended use of animals for this research activity. Significant changes in procedures will not be undertaken without prior notification and approval of the Maryville College IACUC.
- All persons involved in the use of animals on this protocol have been informed of the experimental objectives and methods. Each has received training in the execution of animal-related procedures he/she will perform prior to participation in the protocol, and will participate in any educational or training programs deemed appropriate or necessary by the Maryville College IACUC.
- I agree to follow the provisions of the Animal Welfare Act and the guidelines of the National Institutes of Health on the care and use of laboratory animals.
- I agree to use anesthesia, analgesia and tranquilization to relieve pain or distress whenever use of these agents will not jeopardize the scientific validity of the data. I have specifically consulted with the Maryville College IACUC regarding any experiments that are classified in pain/distress categories C, D, or E.
- I will take appropriate steps to avoid exposure of persons working with these animals to any biohazard agents used in the study.

For any unchecked box above, explain the reason it does not apply.

Purpose of the Study: Briefly describe your proposed research project (or attach a research proposal). Be sure to include a justification for the species and number.

This proposed study will be conducted with the lizard species *Anolis carolinensis*, and the animals used in the experiment will be obtained from laboratory populations rather than the wild. This study will focus on comparative observations based on behavior between two separate populations of *Anolis carolinensis* differentiated by controlled photoperiod and temperature. The animals will be divided into two different populations for the experiment, and each population will consist of six lizards. The purpose of this experiment is to identify behavioral and thermoregulatory characteristics associated with *Anolis carolinensis* in relation to photoperiod and temperature.

Potential Scientific Benefits: State potential value of study with respect to human or animal health, advancement of knowledge, or good of society.

Scientific investigation into an ecological understanding of how species relate and adapt to their environment has important implications in understanding how species may have evolved and how the species interact with their environment. Study of reptilian species, such as lizards, can provide a good understanding of how many species in this taxonomic classification are affected by the characteristics of their environment. *Anolis carolinensis* is an arboreal poikilothermic species and as such, must use its environment to regulate its body temperature; therefore, the ability to thermoregulate using different behaviors is critical for the survival of this species. This study is designed to be implemented in a middle school or high school classroom. This study is designed to introduce students to the scientific concepts of experimental design, animal behaviors, and the way animals interact with their environment.

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