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PHOTOACCELERATION OF EMBRYONIC DEVELOPMENT  
OF QUAIL (*Coturnix coturnix*) UNDER DIFFERENT STIMULI OF  
LIGHT

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## ABSTRACT

In wild birds, incubation period and embryonic development time vary systematically with latitude. Incubation periods tend to be longer at the equator and shorter at higher latitudes. This is a paradox; according to basic developmental and biochemical theory (i.e.,  $Q_{10}$  factors), higher temperatures (low latitudes) should decrease development times, while cooler temperatures (high latitudes) should prolong them. In poultry, incubation periods can be experimentally shortened by exposing eggs to light. The positive influence of light on embryonic growth, called photoacceleration, can begin within hours after an egg is laid and with a low threshold of light intensity (~10 lux). We used photoacceleration to manipulate developmental times at simulated high and low latitudes. To do this, we artificially incubated quail (*Coturnix coturnix*) under two conditions: i) varying photoperiods, and ii) varying wavelengths, and measure heart rate as a surrogate embryonic metabolic rate during light and dark phases. We believe that metabolic/heart rates during both light phases and under blue light will be high enough to account for the differences in incubation periods between birds. We hope to provide support for the testable hypothesis that differences in photoperiod and wavelength may influence variation in the rate of embryonic development in birds.

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## CHAPTER ONE:

### INTRODUCTION AND LITERATURE REVIEW

Developmental plasticity provides an important mechanism for geographic variation in phenotypes (Du *et al*, 2010). The conditions under which an organism grows can alter its development, thus, changing its phenotype later on in life. Avian development is contingent upon numerous zeitgebers with nature. There are five main environmental variables that will influence the rate of embryonic development: temperature, humidity, oxygen and carbon dioxide pressures, and the turning of the egg (Fairchild *et al*, 2000). Embryonic development is a dynamic process that requires a fine balance between these environmental factors (Onagbesan *et al*, 2007). Though it is not included within the traditional list, light has also been postulated to directly influence the rate of development within the embryo. Methods of light treatment such as light schedule, intensity or luminance, and color are important factors that influence avian productivity. Therefore, artificial illumination has been widely used in modern poultry incubation. (Cao *et al*, 2008). To provide a general understanding of avian embryonic development, a knowledge of these variables is necessary.

Gas exchanges are of fundamental importance for embryonic development during incubation and may affect the livelihood of the embryo (Onagbesan *et al*, 2007). The avian eggshell plays an important role in gas exchange. The shell forms the barrier between the internal and external environments. It protects the embryo mechanically against impacts and serves as a physical barrier against bacterial infection. The inner side of the shell also serves as a viable

source of calcium that can be taking up during the development of embryonic bones (Onagbesan *et al*, 2007). The most important feature of the eggshell, however, is its porosity. Romanoff and Romanoff (1949), Wangenstein and Rahn (1970-1971), and Ar *et al*. (1974) all have demonstrated that gases and water vapor are exchanged between the internal and external environments of the egg through the pores in the eggshell according to simple laws of diffusion (Onagbesan *et al*, 2007). Thus, the diffusive properties can increase or decrease depending on the amount of pores located on the shell surface, as well as the physical structure of the pore (i.e. wider, shorter).

Gas exchange between the embryo and the diffused air takes place through specialized and highly vascularized embryonic organs (Onagbesan *et al*, 2007). At discrete points during embryonic development, three different gas exchange mechanisms exist: the area vasculosa, the chorioallantoic membrane, and the lungs (Brown, 2004). The well vascularized area vasculosa fans out from the embryo and surrounds the yolk immediately after fertilization. After the second day, the circulatory system is developing and blood begins to circulate through the embryo and area vasculosa (Brown, 2004). After about the sixth day, the fusion of the chorion and the allantois occurs, and respiratory function transitions to this chorioallantoic membrane (CAM) until the end of incubation. By the eleventh day, the CAM is fully developed, lying attached under most of the inner eggshell membrane and fully functional (Onagbesan *et al*, 2007). The gas flux between the embryo and the environment depends on: i) the gas partial pressures in the blood of the extra-embryonic circulation, ii) the effective gas exchange area, and iii) the thickness and diffusive properties of the material separating the red blood cells from the environment (Onagbesan *et al*, 2007). The outer surface of this structure forms a very dense



capillary bed. Finally, as the developing embryo pips (breaks) the membrane, as well as the outer shell, the chorion begins to breakdown and gas exchange is shifted to the lungs (Brown, 2004).

Oxygen is the gas that drives all of the metabolic machinery within embryonic cells to execute the complex maneuvers of development (Onagbesan *et al*, 2007). During incubation, the oxygen uptake of the egg increases exponentially as the embryo grows rapidly during the first two weeks (Onagbesan *et al*, 2007). Naturally, oxygen supply varies with altitude, as well as latitude. This raised the possibility for the high occurrences of hypoxia during natural incubation. Oxygen supply influences incubation duration and hatchability (Onagbesan *et al*, 2007). Embryonic development is imperative upon the supply, and consumption, of oxygen. Multiple developing organ systems require an adequate supply of oxygen during development. Hypoxia impairs cardiovascular development and function; leading to impaired function of ultimately every other organ system within the embryo (Onagbesan *et al*, 2007). Hypoxia leads to the enhanced transition to pulmonary ventilation, thus, leading to an early hatching of the embryo (Onagbesan *et al*, 2007).

Temperature can affect numerous phenotypic traits in birds. One aspect that is particularly affected by temperature is incubation (Ardia *et al*, 2009). Incubation by parents is extremely energetically expensive. Birds will typically use basal metabolic heat or excess heat generated during non-incubation activities to warm the eggs with minimal direct costs (Ardia *et al*, 2009). The sensitivity of incubation to temperature facilitates experimental manipulations of environmental conditions. Not only do artificially incubating eggs and nests mimic the heat generated by parental birds, but it shows a reduction in the energetic constraints during the incubation period. Thus, this would allow for a redistribution of nutrients into a greater investment in embryonic development (Ardia *et al*, 2009). Thereby, ambient temperatures can

cause changes within incubation, demonstrating the phenotypic effect on birds. Any increase in incubation temperature can increase the rate of development. Thus, specific mechanisms to accelerate embryogenesis might include an acceleration of heart rate relative to temperature (Du *et al*, 2010). Avian embryos do have the ability to sense external environments and adjust their development independent of maternal effects in eggs (Clark *et al*, 2012).

The pace of life is believed to increase with respect to latitude for almost all animals (Cooper *et al*, 2011). Avian incubation periods have been shown to shorten as the general pace of life quickens with distance from the equator. Differences in the latitudinal photoperiod may actually influence the variation in the rate of embryonic development of ectotherms through a process known as photoacceleration (Cooper *et al*, 2011). Photoperiod is an arrantly reliable component of the natural environment that varies predictably with latitude; playing a crucial role within embryonic growth and development. The rate and mechanism of photoacceleration varies with stage of embryo development (Cooper *et al*, 2011). The fastest rate of embryonic development occurs with light stimulation. The physiological mechanism by which light stimulates growth relies on the two primary components of the avian circadian rhythm, the hypothalamic pacemaker and the pineal gland (Cooper *et al*, 2011). Light can also penetrate to the cellular level, activating cytochromes in the mitochondrial electron transport chain. Light can regulate cellular metabolism by the means of cAMP, subsequently leading to the initiation of DNA synthesis; and ultimately the influence of gene expression within embryonic development (Cooper *et al*, 2011). With respect to most animals, light suggests cues for the maturation of the internal circadian rhythm, birds being no different. Lastly, light exposure causes changes in embryonic metabolic rate.

The pineal gland represents one of the most important components within the circadian rhythm in birds. This gland plays an important role, in conveying information about the prevailing photoperiod to the internal milieu of an organism (Zeman *et al*, 2004). Avian systems are constantly exposed to fluctuating rhythmic temperature changes, either from the direct environment or from changes in body temperature of the incubating parent. Ambient temperature is a crucial variable that actually synchronizes normal bodily functions in a 24-hour period (Ardia *et al*, 2009, Zeman *et al*, 2004). Rhythmic melatonin production occurs during the last third of avian development, however, at that time a normal functioning circadian rhythm is already clearly discernible (Zeman *et al*, 2004). The circadian rhythm of the developing embryo can be synchronized by numerous factors: by the incubating parent, cycles of light and darkness exposure, as well as temperature regimes (Zeman *et al*, 2004). Thus, the embryo may be informed of its external environment much before it hatches.

Melatonin is a hormone secreted by the pineal gland that modulates sleep patterns due to seasonal functions. In birds, melatonin is the most important connector of the internal circadian rhythm to physiological functions (Brown, 2004). Melatonin production is able to synchronize with the illumination cycle (Akasaka *et al*, 1995, Zeman *et al*, 2004). Thus, suggesting that pineal cells contain both a circadian oscillator and a photoreceptor, and that the cycle of melatonin synthesis and release are generated by these oscillations of light (Akasaka *et al*, 1995). Melatonin is directly involved in the adaptation of retinal photoreceptors and skin melanophores to changing intensity of ambient light (Brown, 2004). Melatonin release is increased in dark phased, and significantly decreased in light phases. This is a clear indication that the synthesis is already regulated by photic information all throughout avian development, probably via a photoreceptor (Akasaka *et al*, 1995). Melatonin synthesis is tightly regulated by both the

photoperiod and circadian oscillators (Akasaka *et al*, 1995). Cyclic AMP is an important regulator of circadian rhythm; showing a strong correlation between the effects of light on melatonin, and ultimately, the circadian rhythm of birds.

Circadian rhythms and metabolism are closely linked together in birds. The metabolic pattern of avian development is unique among all vertebrates. Avian embryos depend upon external sources of heat to grow until development establishes homothermy (Cooper *et al*, 2011). Metabolism in embryos is often times defined as the sum total of chemical reactions that take place within the body. The rate at which these processes occur is directly correlated to an increase in temperature, thus, metabolic activity of organisms are linked to its internal body temperature (Greenwald and Kanter, 1979). Metabolic rate, defined as energy metabolism per unit time, can be determined in several ways (Brown, 2004). First, it can be directly determined by measuring heat production. A more indirect way can be measured through food intake and waste excretion. Lastly, metabolism can be determined by measuring an animal's gas exchange. Heart rate and metabolic activity are directly coupled in all animals. Therefore, a change in metabolic rate would lead to a change in heart rate, since heart rate supports the metabolic rate of the organism by supplying metabolic needs to the developing embryo (Pearson *et al.*, 1999).

The avian embryo is ideal for measuring heart rate for numerous reasons. Avian embryos do have the ability to sense external environments and adjust their development independent of maternal effects in eggs (Clark *et al*, 2012). Embryos develop within a hard-cased eggshell, completely independent upon their maternal body, making their physiological needs completely uninfluenced by the functions of their mother. Thus, the patterns of heart rate in embryos originate from their own cardiac pacing activities (Brown, 2004). Secondly, the eggshell allows for the measurement of heart rate, without disturbing other metabolic processes (Brown, 2004).

Cardiac output is an important determinant of the rate of embryogenesis because it plays a critical role in nutrient and oxygen delivery during development.

Heart rate tends to increase asymptotically with development early on in the avian embryo. Thus, the rate of the heart beat has long been used as an indicator of the functioning of the organ itself, and, indirectly of other normal and pathological conditions within the embryo (Romanoff, 2005). As with any developing embryo, avian heart rate changes quite regularly, as well as drastically. These changes are directly comparable, and reflect the changing metabolic requirements of the embryo. All avian embryos are ectothermic up until hatching; however, the development of endothermy starts with these increases in metabolic demand (Pearson *et al*, 1998). Therefore, making any shift in thermoregulation an influence to the embryonic heart beat of the organism.

## OBJECTIVES AND HYPOTHESES

The main objective was to investigate changes in embryonic heart rate, as a result of increasing metabolic activity, across the developmental period of birds. Two experiments, varying light length and wavelength, will be utilized and compared to one another. we used light as an external cue, or zeitgeber, to entrain metabolic responses at different stages of development. We were specifically interested in understanding the way in which development might be altered by natural latitudinal changes in light:dark (L:D) cycles.. There is evidence that this occurs in the wild, producing longer developmental periods near the equator and shorter developmental periods at higher latitudes. Secondly, we will be looking specifically at the

varying effects wavelength plays on avian development. Eggs reared under blue light should produce metabolic changes that are more significant than eggs reared under the wavelengths that are higher (red light) or lower (ultraviolet light). It was hypothesized that blue light will produce changes that should be comparable to those found within the all white light control, or those in the temperate latitude light cycle. We will test the hypothesis that light cycle governs these latitudinal patterns as follows:

### **Experiment 1:**

$H_0$ : If there is a direct correlation between latitudinal light cycle and changes in development time we expect to see changes in both metabolism and heart rate in response to variation in artificial light cycles. This would suggest that gene regulation during development exhibits a plastic response to light. To specifically test this hypothesis, we will manipulate the following environmental factors during development:

- a. Light cycle (will be varied; 4 treatments)
- b. Temperature (held constant)
- c. Humidity (held constant)

$H_A$ : The lack of any light-induced change in embryonic development time would suggest that metabolism/heart rate is genetically constrained. To further explore this possibility, two alternate hypotheses will be tested:

- d. Mean embryonic heart rate incubated under complete darkness (D:D) will not differ from heart rate compared to the other groups within the study.

- e. Heart rate between the different varying light patterns will not differ between groups.

### **Experiment 2:**

$H_0$ : If there is a direct correlation between light wavelength and changes in development time we expect to see changes in both metabolism and heart rate in response to variation in light wavelengths. This would suggest that gene regulation during development exhibits a plastic response to light. To specifically test this hypothesis, we will manipulate the following environmental factors during development:

- a. Wavelengths (will be varied; 3 treatments)
- b. Temperature (held constant)
- c. Humidity (held constant)

$H_A$ : The lack of any wavelength-induced change in embryonic development time would suggest that metabolism/heart rate is genetically constrained. To further explore this possibility, two alternate hypotheses will be tested:

- d. Mean embryonic heart rate incubated under white (fluorescent) light will not differ from heart rate compared to the other groups within the study.
- e. Heart rate between the different varying light wavelength patterns will not differ between groups.

## **CHAPTER TWO:**

### **MATERIALS AND METHODS**

#### Source and Incubation of Eggs

To test the hypotheses about the photoacceleration of avian development, fertilized quail (*Coturnix coturnix*) eggs were obtained from Murray McMurray Hatchery in Webster City, IA, and shipped to our laboratory at Penn State Erie, the Behrend College. In the lab, eggs were held below physiological temperature (37°C) to prevent any development from occurring until placement in incubators. On day one of incubation, eggs will be placed in one of four incubators held at a constant relative humidity (75±5%) and temperature (36.5±0.27°C). A thermometer that was placed inside each of the incubators measured temperature. The eggs were placed in an automatic egg turner with a complete revolution of 30 degrees every 4 hours. An embryo was considered 'Day 1' after it was in incubation for a full twenty-four hours.

Each incubator was set up to simulate a different light:dark cycle, or wavelength treatment. The treatments were no light (0 L – control group, D:D), equatorial (12 L: 12 D – experimental group 1), temperate (18 L: 6 D – experimental group 2), arctic (24 L) for experiment 1. Experiment 2 treatments will be red LED light (RL), blue LED light (BL), and ultraviolet LED light (UV), using white (40 watt full spectrum fluorescent) light (WL) as an endogenous control group. Eggs were randomly assigned to the length, or treatment, of light during the incubation period. Broken eggs were still utilized, but noted as broken to avoid any outgroup within the experiment. Damaged eggs were positioned away from direct light sources



to allow viable eggs the greatest chance to absorb the light. Incubators were larger Styrofoam containers that contained large, insulated plexi-glass windows to place lights directly over top (Hova-Bator Incubation – Model Number: 2362N). Incubators contained fans directly inside the lid that allowed for constant airflow, and control of humidity. Incubator ventilation is designed to provide adequate oxygen to the embryo and eliminate excessive carbon dioxide from building up.

For experiment 1, eggs (N=105) were incubated under various light regiments throughout the entire incubation period of embryonic development. On day 7-8 of incubation, an EKG routine was introduced. For experiment 2, eggs (N=147) were incubated under a particular constant light wavelength throughout the entire incubation period . On day 7-8 of incubation, an EKG routine was introduced.

#### Egg Weight Measurement

Egg mass (in grams) was measured on an OHAUS scale (Model PS 121) to within 0.1 g immediately after being taken out of the incubator. Measurements were taken immediately before being placed in the incubator, marked as Day 0, and then were taken every other day throughout the entire incubation period. The scale was allowed to stabilize after each egg was placed on it. Recordings were put in an Excel spreadsheet for further use.

## EKG Measurement

### *EKG Placement*

A total of 5-7 eggs per treatment were used to test the heart rate of the developing embryo. An egg candler was used to determine vascularization and position of the developing embryo inside of the eggshell. A dissecting probe was used to put three small holes about 1 mm in diameter into the shell. The holes were placed into the egg in the shape of an equilateral triangle on the side of the egg ~2 cm apart to allow for the best electrocardiogram activity. Silicon gel was used to seal the hole and to fix the electrode in place with minimal reduction of the diffusive surface area of the egg. Three small needles were inserted into the egg using the same 3-lead wire EKG system (Einthoven's Triangle) typically used in human EKG's. The electrodes were connected to a Biopac Systems, Inc monitor (MP 35) to record and measure the heartbeat of each embryo. EKG time lengths varied throughout the incubation period. As the incubation period increased, EKG lengths decreased. Starting time was held constant at 200 seconds and never went below 120 seconds in length. Measurements were run every other day, starting at day 7-8, for a total of seven readings per egg.

### *EKG Set-up*

Embryonic heart rate was detectable invasively using an electrocardiogram measuring system that was original to this experiment. A *floating* platform was used to support the egg, consisted of a clay triangle resting atop of an O-ring, was attached to a ring stand. The platform attenuated most of the external vibrations that would contaminate the readings of the electrocardiogram. The egg was placed atop the clay triangle with the equilateral triangle of 1 mm holes positioned facing downward, through the triangle. The electrocardiogram needles were

inserted from the bottom of the egg up through the holes, gently bringing them into contact with the vasculature contained within the embryo. Needles were inserted as to not disturb the developing vascular tissue of the embryo.

### EKG Protocol

#### a) Control Group

The control group consisted of embryos exposed to total darkness (D:D) throughout their entire incubation, including the measurement period (with the exception of two to three minutes required for electrocardiogram every other day starting at day 7). The electrode wires were connected to the Biopac Systems, Inc monitor (MP35). The program was used to record heart rate, which was stored in data files on the computer. Measurements of electrocardiogram were made every other day starting at day 7-8 and continued till the incubation period was over. Files were stored on a personal computer for heart rate analysis.

#### b) Experimental Group

The experimental group consisted of exposures to both varying light treatments and wavelengths (18L:6D, 12L:12D, 24L, red light, blue light, UV light). All equipment was set-up the same as the D:D electrocardiogram system described above. The experimental groups from Experiment 1 were exposed to light from a 40-watt full spectrum tube that was set on a timer to turn on and off at the treatment's appropriate time. Experimental groups from Experiment 2 were exposed to constant light that contained a specific light spectrum. Temperature was held steady over the entire heart rate measurement period. Files were stored on a personal computer for heart rate analysis.

## CHAPTER THREE:

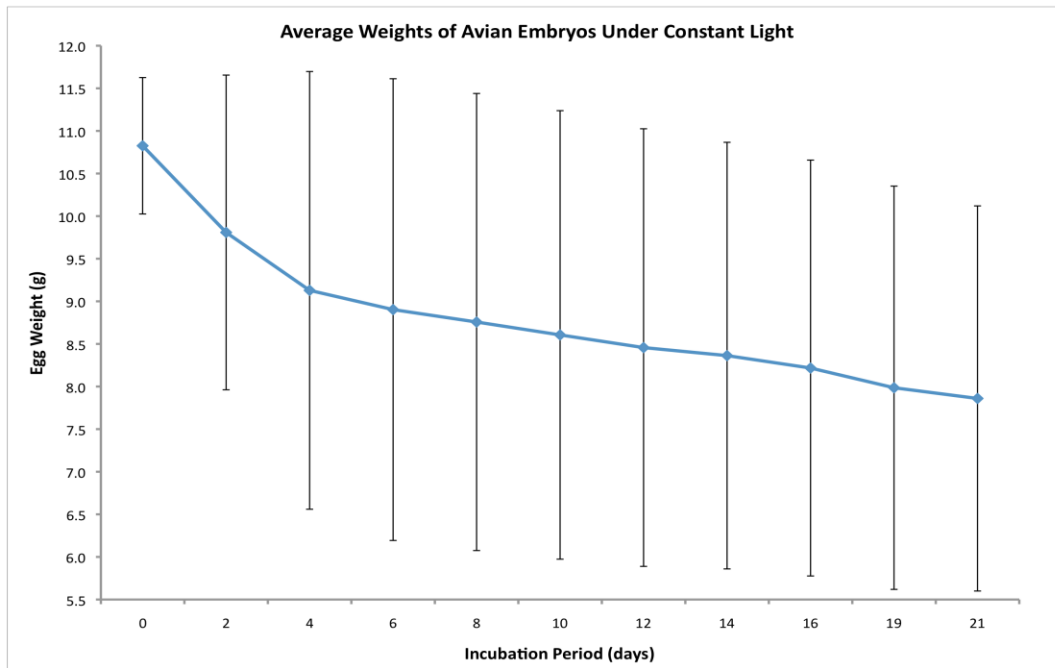
### RESULTS

A total of 252 eggs were received and assigned to incubators (approximately 35-45 eggs per incubator, 126 per experiment) through two different experiments. Results from both experiments are shown below as averaged weights per day for the entire incubation period. Data from eggs that were damaged or broken throughout the incubation period were throw out to not give faulty data, or large standard deviations.

#### Effects of Light on Egg Mass

##### *Effects of Varying Length of Light on Embryo Development*

Developmental time of embryogenesis of quail was tested under various light systems (Figures 1-4). The correlation between latitudinal light cycles and changes in development time helped suggest that gene regulation during development exhibits a plastic response to light. Results show that egg development is positively correlated to the amount of available ambient light. Development increased, thus, metabolic rates were increasing with increased light amount. Eggs showed the most drastic reductions in mass under constant light, further supporting our hypothesis (Figure 1). Larger than normal error bars may be attributed to light not evenly spreading within the incubators.



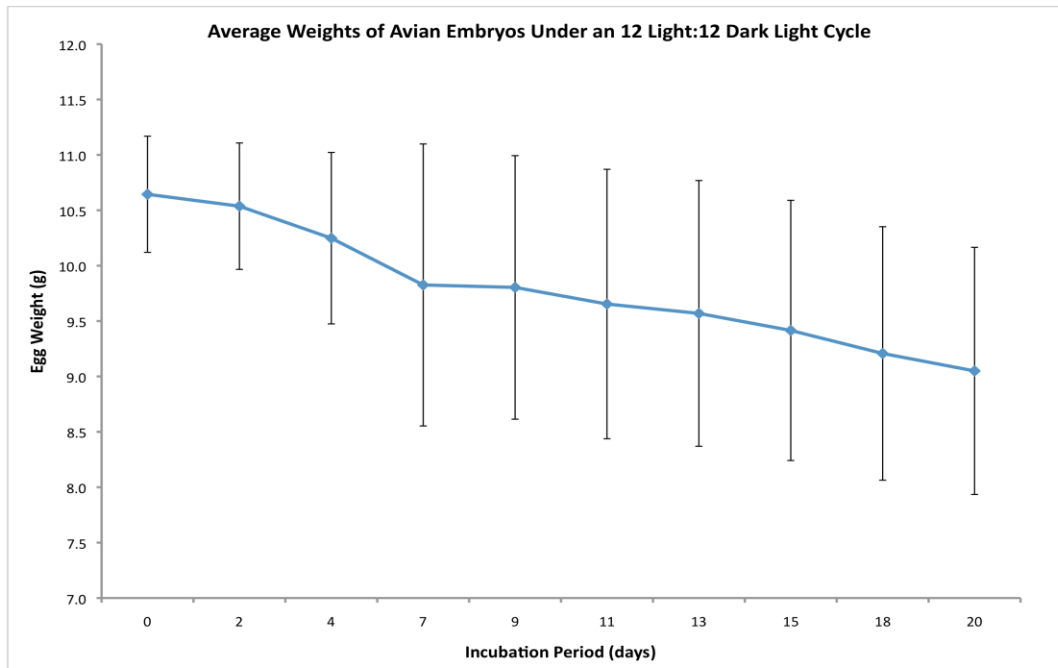
**Figure 1: Average Weights of Avian Embryos Under Constant Light.** Quail eggs were incubated under constant ambient light for the duration of the incubation period. Each data point represents the average weight of treated eggs. Incubators were kept at a relative humidity ( $75\pm 5\%$ ) and constant temperature ( $36.5\pm 0.27^\circ\text{C}$ ). Error bars indicate the standard deviation of the mean that was computed.

In comparison, the ability of eggs to fully develop was tested under a temperature latitude light scheme. Eggs incubated under the most drastic, experimental latitude, 18L:6D, showed comparable results to the light control. Temperate treated egg masses decreased significantly during the incubation period. Eggs showed reductions in mass that seemed to resemble the same reduction seen within constant light (Figure 2). With any experiment, there exists variation between all eggs, but it's to be noted the same general trend in reduction is seen within the temperate experimental group. Larger than average error bars may be attributed to eggs being in different states upon receiving them.

**Figure 2: Average Weights of Avian Embryos Under A Temperate Light Cycle (18L:6D).** Quail eggs were incubated under an ambient light cycle that represented a temperate latitude for the duration of the incubation period. Each data point represents the average weight of treated eggs. Incubators were kept at a relative humidity ( $75\pm 5\%$ ) and constant temperature ( $36.5\pm 0.27^\circ\text{C}$ ). Error bars indicate the standard deviation of the mean that was computed.

In contrast, as latitudinal changes tend to get more toward the equator, egg mass is shown to not reduce as significantly. The metabolic rate slows down due to darkness, thus, reducing the development of the embryo. Egg mass tends to stay on the heavier end of the spectrum due to the increasing darkness. Compared to the all-light control, weight reduction was decreased (Figure 3). A small amount of variation is seen between average egg masses between the two experimental groups showing that the metabolic rate is slower. The general trend shows a slightly slower, more general reduction in egg mass, representing a reduced developmental

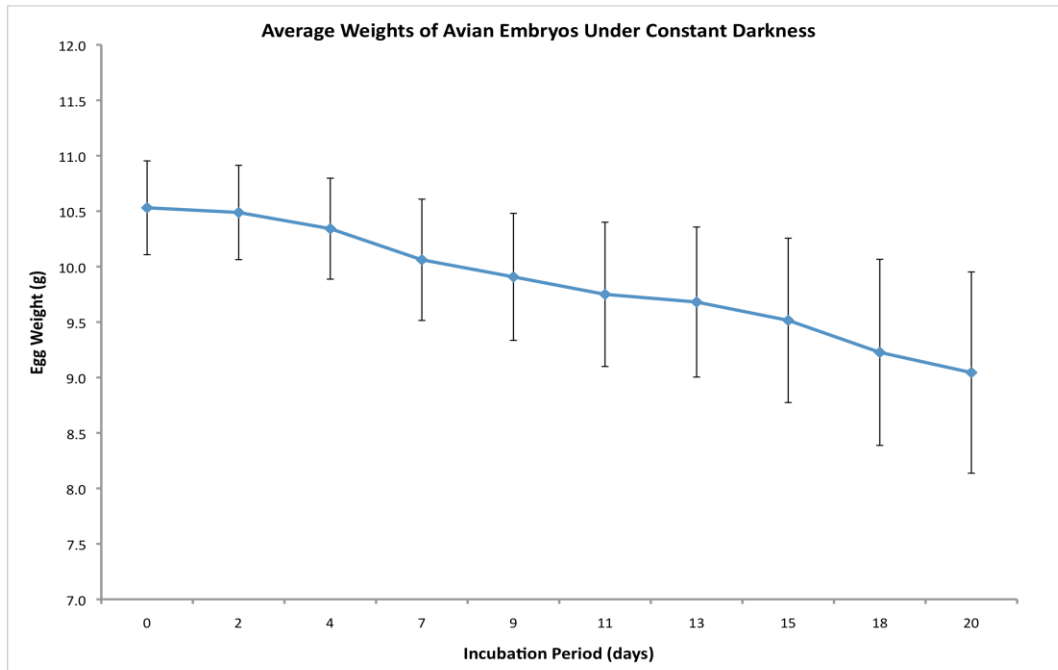
ability.



**Figure 3: Average Weights of Avian Embryos Under A Equatorial Light Cycle (12L:12D).** Quail eggs were incubated under an ambient light cycle that represented an equatorial latitude for the duration of the incubation period. Each data point represents the average weight of treated eggs. Incubators were kept at a relative humidity ( $75\pm 5\%$ ) and constant temperature ( $36.5\pm 0.27^\circ\text{C}$ ). Error bars indicate the standard deviation of the mean that was computed.

Results of the dark control show that development was slowed down throughout the entire incubation period. As darkness levels increased, egg mass reduction tended to slow down significantly. The general trend line is very gradual over the incubation period, representing a slower metabolic rate within the developing eggs. Reductions in mass were significantly smaller in comparison to its counterpart control, constant light. Weights tended to level off for a few days, followed by a slight drop in mass and then level off once again. This trend can be seen

throughout the entire incubation period (Figure 4).

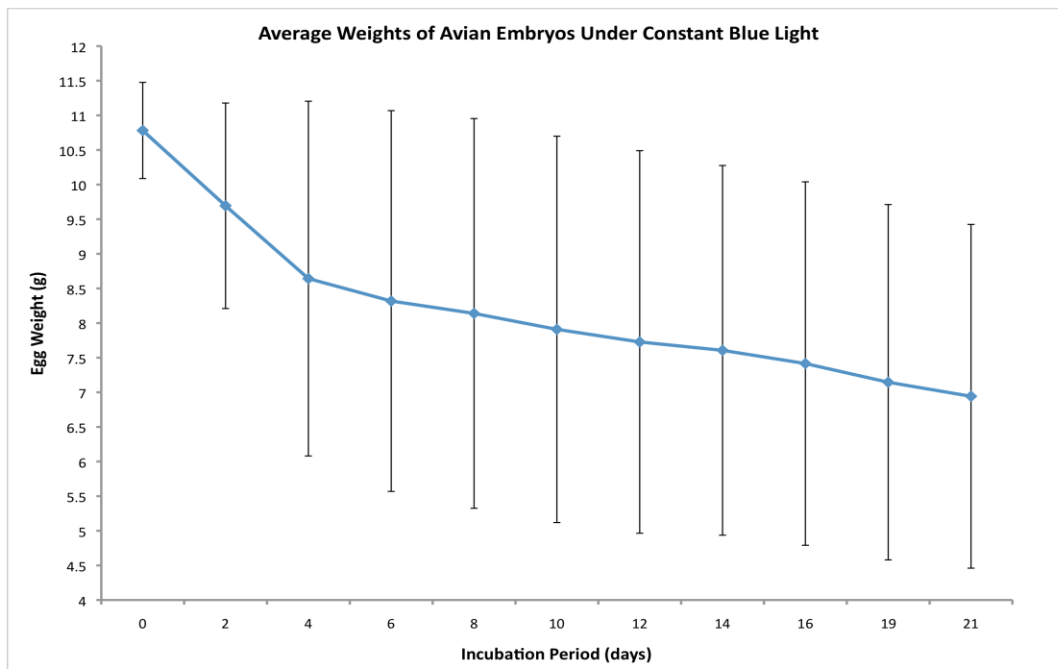


**Figure 4: Average Weights of Avian Embryos Under Complete Darkness.** Quail eggs were incubated under complete darkness for the duration of the incubation period. Each data point represents the average weight of treated eggs. Incubators were kept at a relative humidity ( $75\pm 5\%$ ) and constant temperature ( $36.5\pm 0.27^\circ\text{C}$ ). Error bars indicate the standard deviation of the mean that was computed.



### *Effects of Varying Wavelengths on Embryo Development*

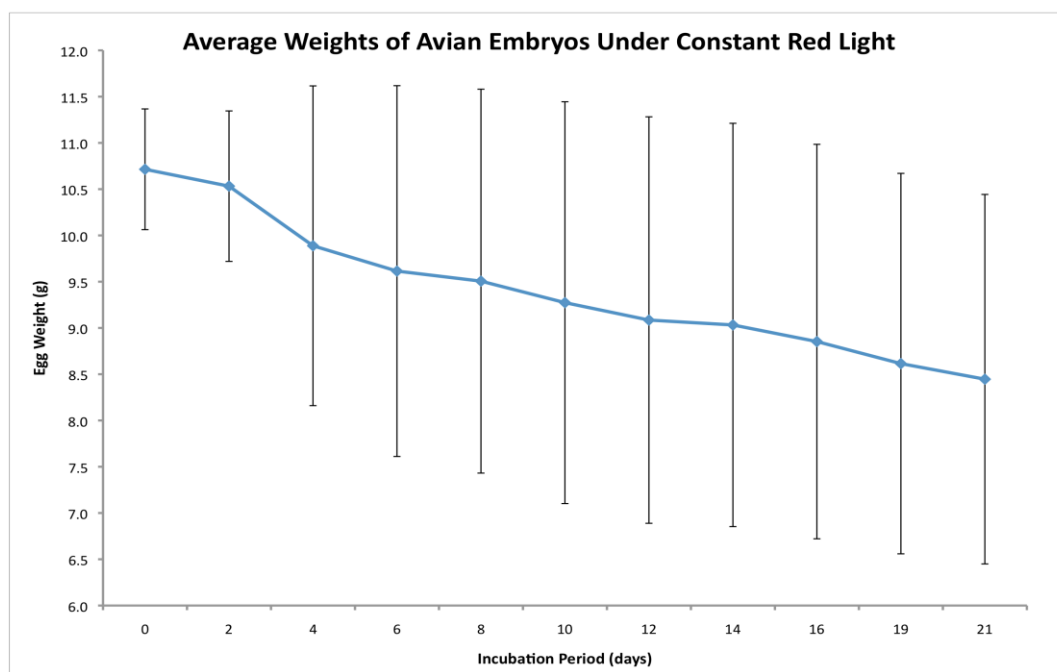
The ability of avian eggs to grow and develop under various wavelengths was tested (Figures 5-7). Red, blue, and ultraviolet lights were used to test for the metabolic response due to varying artificial wavelengths. Results show that avian growth was best when reared under constant blue light, while slower development was seen in eggs reared under ultraviolet and red light. Eggs reared under blue light had significantly reduced egg mass at the end of the incubation period (Figure 5). Even when compared to the all-light control, eggs consistently weighed less on average. Large error bars can be attributed to the uneven distribution of the monochromatic blue light within the incubator.



**Figure 5: Average Weights of Avian Embryos Under Constant Blue Light.** Quail eggs were incubated under a constant blue light for the duration of the incubation period. Each data point represents the average weight of treated eggs. Incubators were kept

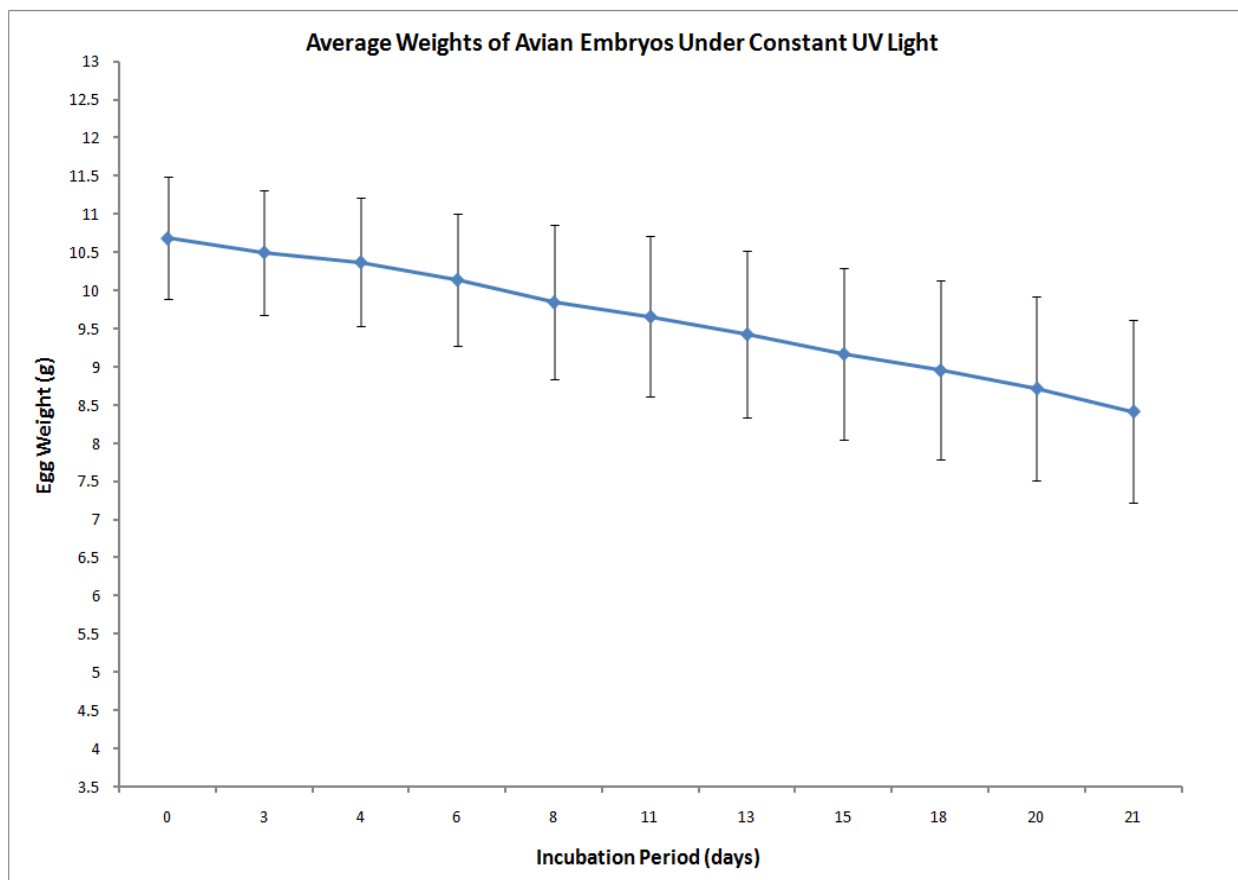
at a relative humidity ( $75\pm 5\%$ ) and constant temperature ( $36.5\pm 0.27^\circ\text{C}$ ). Error bars indicate the standard deviation of the mean that was computed. .

Eggs reared under red light and ultraviolet showed similar growth patterns throughout the incubation period. Both treatments resulted in the same reduction in egg mass over the period. Red light, however, showed a more, gentle slope with mass. Eggs incubated in red light had more reduction when compared to the all-dark control treatment, yet, didn't have the same drastic slope that was seen in the eggs that were reared in all light (Figure 6). Large error bars may be due to the monochromatic light, and the uneven distribution of light within the incubator.



**Figure 6: Average Weights of Avian Embryos Under Constant Red Light.** Quail eggs were incubated under constant red light for the duration of the incubation period. Each data point represents the average weight of treated eggs. Incubators were kept at a relative humidity ( $75\pm 5\%$ ) and constant temperature ( $36.5\pm 0.27^\circ\text{C}$ ). Error bars indicate the standard deviation of the mean that was computed.

Ultraviolet, or UV, light incubated eggs showed similar reductions in egg mass to red light, but had a much more drastic slope throughout the entire incubation period (Figure 7). Eggs reared under UV light had a constant reduction in egg mass throughout the entire incubation period. Average weights showed a relatively linear relationship between UV light and egg mass reduction. Large error bars may also be attributed to uneven distribution of the monochromatic light within the incubator.



**Figure 7: Average Weights of Avian Embryos Under Constant UV Light.** Quail eggs were incubated under constant Ultraviolet light for the duration of the incubation period. Each data point represents the average weight of treated eggs. Incubators were kept at a relative humidity ( $75\pm 5\%$ ) and constant temperature ( $36.5\pm 0.27^\circ\text{C}$ ). Error bars indicate the standard deviation of the mean that was computed.

## Effects of Light on Heart Rate

To test the embryonic heart rate throughout the entire incubation period, six eggs from each of the treatments were taken and electrocardiograms were performed on each egg every other day for a total of seven electrocardiogram (EKG) analyses. Of the six eggs, only some showed clear signs of a beat that was able to be interpreted. On viable EKG's, the heart beat was found by highlighting one single beat (i.e one P-P wave, or one R-R wave) and determining the beats per minute. This was done using triplicates on each EKG and then the three were averaged together to give an average beat per minute (BPM) for each appropriate egg. For treatments that produced more than one egg that showed a well pronounced beat, the BPM of each egg was averaged. The average BPM for each treatment is shown in Table 1.

**Table 1: Average Beats per Minute for Each Treatment**

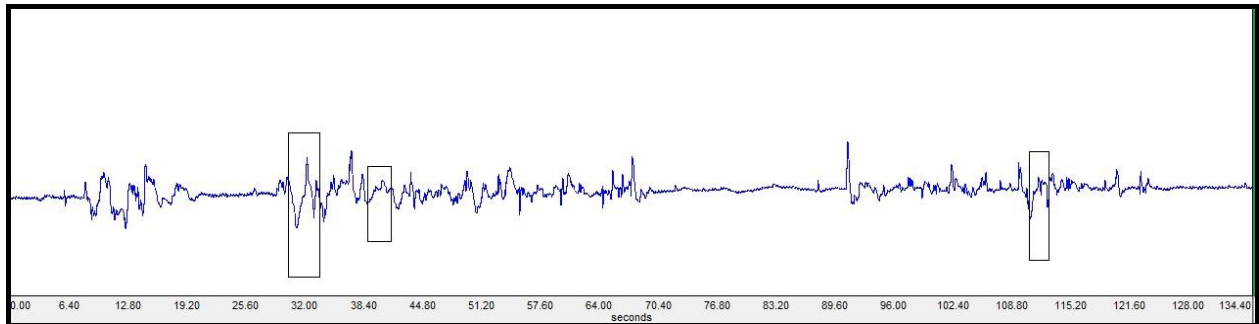
<b>Light Treatment</b>	<b>Average BPM</b>
24 Light	36.94492
18:6 Light	46.37186
12:12 Light	20.48898
24 Darkness	14.39043
Red	16.32479
Blue	29.6330
UV	20.23647

**Table 1: Average Beats per Minute for Each Treatment.** Heart beat was found using selected EKG's that showed pronounced heart rates throughout the entire EKG trial. Heart rates were found using triplicates on the same electrocardiogram and then averaged to determine appropriate heart rate for the individual egg. Treatments that produced multiple viable eggs had the individual BPM averaged together to give a single comparable number.

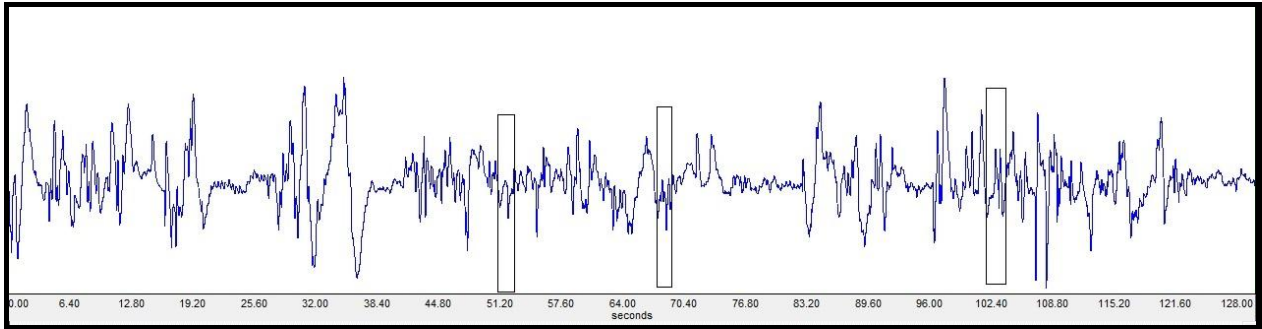
Selected electrocardiograms are shown in Figures 8-26.

### *Effects of Length of Light on Heart Rate*

Both figure 8 and figure 9 are from one individual egg from the all light control group. Figure 8 shows the heart rate from an early day, while figure 9 shows the heart rate of the egg on the last day of incubation. EKG's were run on a max of three minutes, and tended to get shorter throughout the incubation period. Boxes placed on the electrocardiograms represent the single beats that were used to determine the individual heart rate. The triplicates were analyzed and averaged together to give an average BPM for each selected EKG. Average BPM for the selected egg from the all light control was 30.52353 and 43.36631, respectfully; yielding an average of 36.94492 BPM.

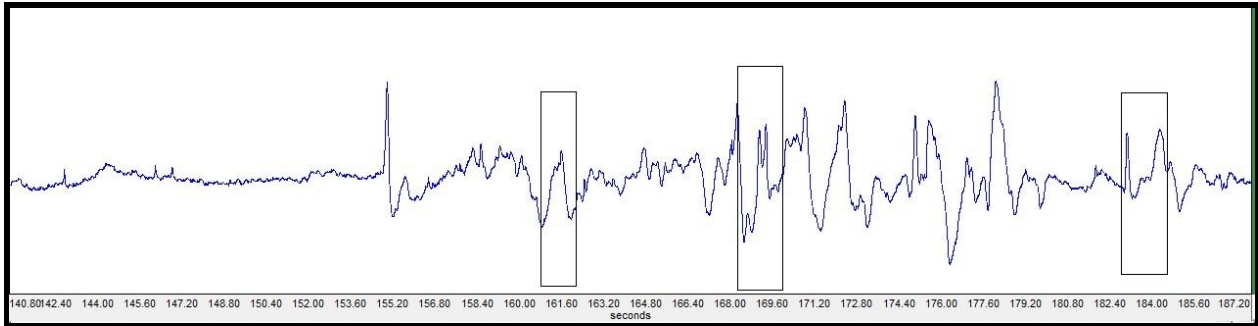


**Figure 8: Electrocardiogram of an Individual Egg Incubated Under Constant Light.** EKG's were run on select eggs from the treatment. Eggs were incubated under constant ambient light for the entire incubation period. EKG's that showed clear, visible signs of a heart beat were chosen and average beats per minute (BPM) were determined using single beats (one P-P wave or one R-R wave). This was replicated three times on each viable egg, and then the BPM's were average to yield a single average BPM for the egg. Boxes represent the single beats that were selected to determine BPM. Average BPM was determined to be 30.52353.

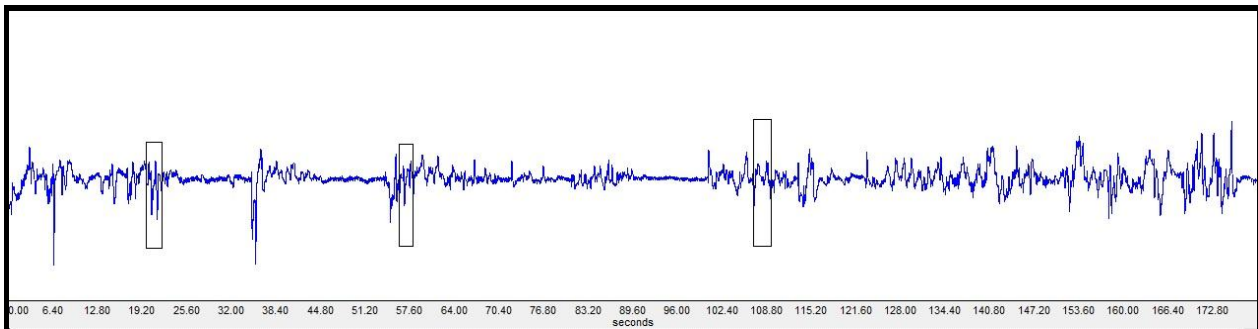


**Figure 9: Electrocardiogram of an Individual Egg Incubated Under Constant Light.** EKG's were run on select eggs from the treatment. Eggs were incubated under constant ambient light for the entire incubation period. EKG's that showed clear, visible signs of a heart beat were chosen and average beats per minute (BPM) were determined using single beats (one P-P wave or one R-R wave). This was replicated three times on each viable egg, and then the BPM's were average to yield a single average BPM for the egg. Boxes represent the single beats that were selected to determine BPM. Average BPM was determined to be 43.36631.

Figure 10 and figure 11 are from an individual egg from the temperate light (18L:6D) group. Figure 10 shows the heart rate from an early day, while figure 11 shows the heart rate of the egg on the last day of incubation. EKG's were run on a max of three minutes, and tended to get shorter throughout the incubation period. Boxes placed on the electrocardiograms represent the single beats that were used to determine the individual heart rate. The triplicates were analyzed and averaged together to give an average BPM for each selected EKG. Average BPM for the selected eggs was 38.29368 and 54.45004, respectfully; yielding an average of 46.37186 BPM.



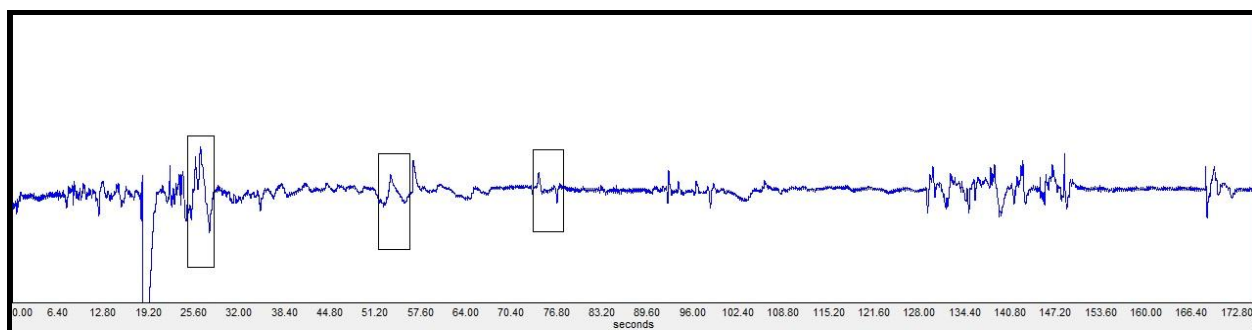
**Figure 10: Electrocardiogram of an Individual Egg Incubated Under Temperate Light Cycle.** EKG's were run on select eggs from the treatment. Eggs were incubated under an equatorial light cycle for the entire incubation period. EKG's that showed clear, visible signs of a heart beat were chosen and average beats per minute (BPM) were determined using single beats (one P-P wave or one R-R wave). This was replicated three times on each viable egg, and then the BPM's were average to yield a single average BPM for the egg. Boxes represent the single beats that were selected to determine BPM. Average BPM was determined to be 38.29368.



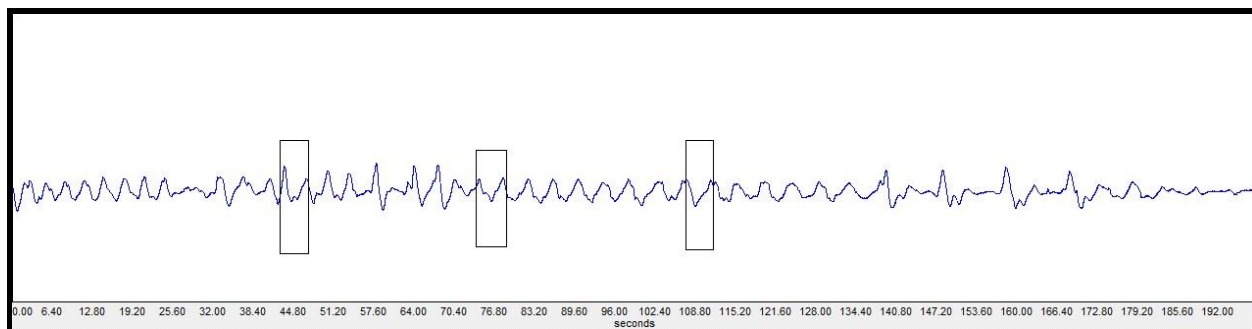
**Figure 11: Electrocardiogram of an Individual Egg Incubated Under Temperate Light Cycle.** EKG's were run on select eggs from the treatment. Eggs were incubated under a temperate light cycle for the entire incubation period. EKG's that showed clear, visible signs of a heart beat were chosen and average beats per minute (BPM) were determined using single beats (one P-P wave or one R-R wave). This was replicated three times on each viable egg, and then the BPM's were average to yield a single average BPM for the egg. Boxes represent the single beats that were selected to determine BPM. Average BPM was determined to be 46.37186.

Figure 12, figure 13, and figure 14 are from individual eggs from the equatorial light (12L:12D) group. All three figures show separate eggs, all incubated in the same experimental condition. EKG's were run on a max of three minutes, and tended to get shorter throughout the incubation period. Boxes placed on the electrocardiograms represent the single beats that were

used to determine the individual heart rate. The triplicates were analyzed and averaged together to give an average BPM for each selected EKG. Average BPM for the selected eggs was 19.20844, 14.64739 and 27.61111, respectfully; yielding an average of 20.48868 BPM.

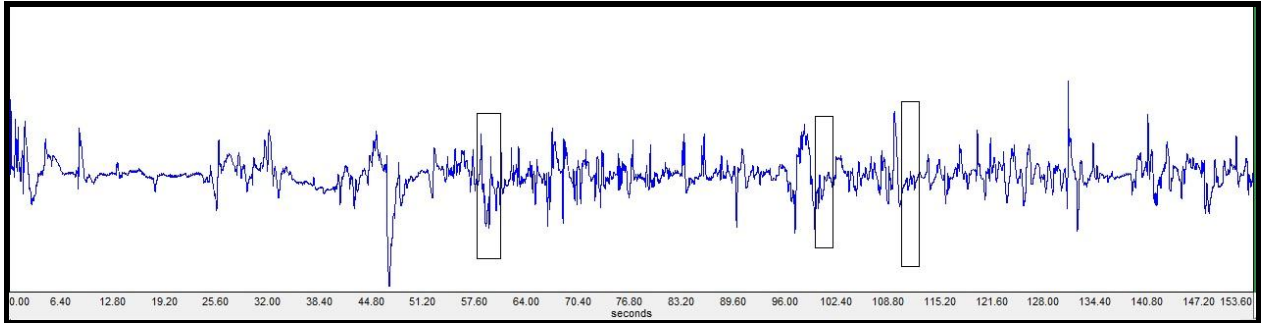


**Figure 12: Electrocardiogram of an Individual Egg Incubated Under an Equatorial Light Cycle.** EKG's were run on select eggs from the treatment. Eggs were incubated under an equatorial light cycle for the entire incubation period. EKG's that showed clear, visible signs of a heart beat were chosen and average beats per minute (BPM) were determined using single beats (one P-P wave or one R-R wave). This was replicated three times on each viable egg, and then the BPM's were average to yield a single average BPM for the egg. Boxes represent the single beats that were selected to determine BPM. Average BPM was determined to be 19.20844.



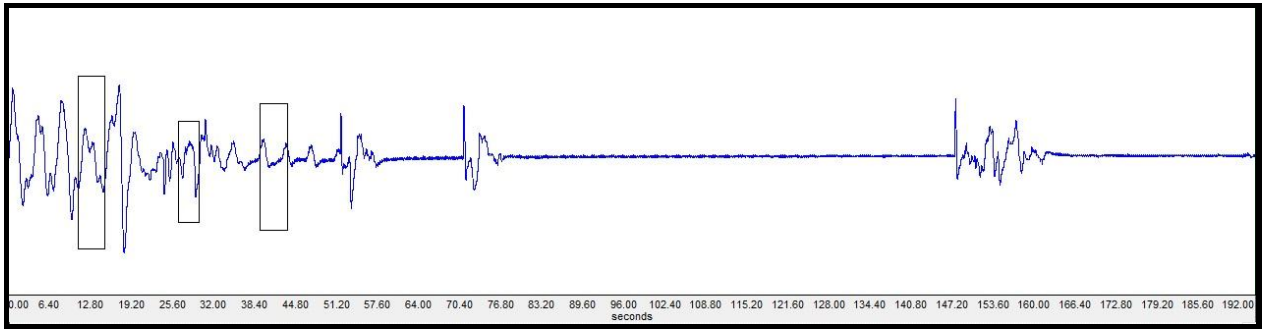
**Figure 13: Electrocardiogram of an Individual Egg Incubated Under an Equatorial Light Cycle.** EKG's were run on select eggs from the treatment. Eggs were incubated under an equatorial light cycle for the entire incubation period. EKG's that showed clear, visible signs of a heart beat were chosen and average beats per minute (BPM) were determined using single beats (one P-P wave or one R-R wave). This was replicated three times on each viable egg, and then the BPM's were average to yield a single average BPM for the egg. Boxes represent the single beats that were selected to determine BPM. Average BPM was determined to be 14.64379.



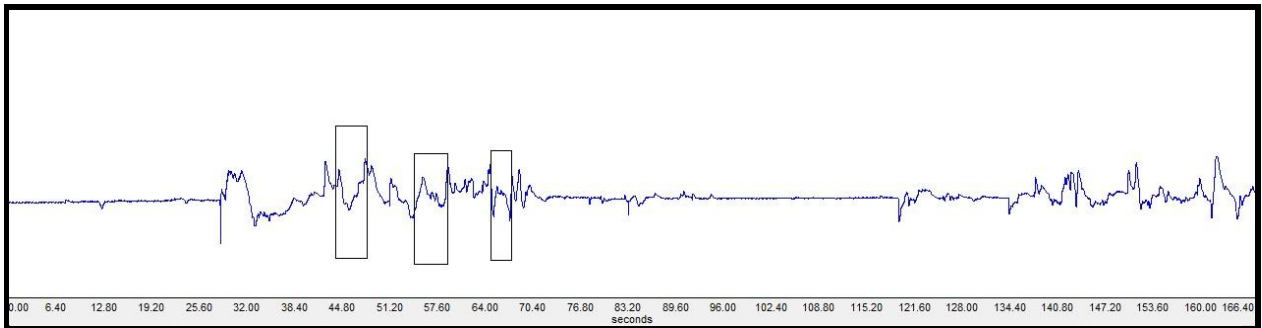


**Figure 14: Electrocardiogram of an Individual Egg Incubated Under an Equatorial Light Cycle .** EKG's were run on select eggs from the treatment. Eggs were incubated under an equatorial light cycle for the entire incubation period. EKG's that showed clear, visible signs of a heart beat were chosen and average beats per minute (BPM) were determined using single beats (one P-P wave or one R-R wave). This was replicated three times on each viable egg, and then the BPM's were average to yield a single average BPM for the egg. Boxes represent the single beats that were selected to determine BPM. Average BPM was determined to be 27.61111.

Figure 15 and figure 16 are from individual eggs from the complete darkness control group. Both figures show separate eggs, incubated in the same experimental condition. EKG's were run on a max of three minutes, and tended to get shorter throughout the incubation period. Boxes placed on the electrocardiograms represent the single beats that were used to determine the individual heart rate. The triplicates were analyzed and averaged together to give an average BPM for each selected EKG. Average BPM for the selected eggs was 15.14962 and 13.63125, respectfully; yielding an average of 14.39043 BPM.



**Figure 15: Electrocardiogram of an Individual Egg Incubated Under Complete Darkness.** EKG's were run on select eggs from the treatment. Eggs were incubated under complete darkness for the entire incubation period. EKG's that showed clear, visible signs of a heart beat were chosen and average beats per minute (BPM) were determined using single beats (one P-P wave or one R-R wave). This was replicated three times on each viable egg, and then the BPM's were average to yield a single average BPM for the egg. Boxes represent the single beats that were selected to determine BPM. Average BPM was determined to be 15.14962.



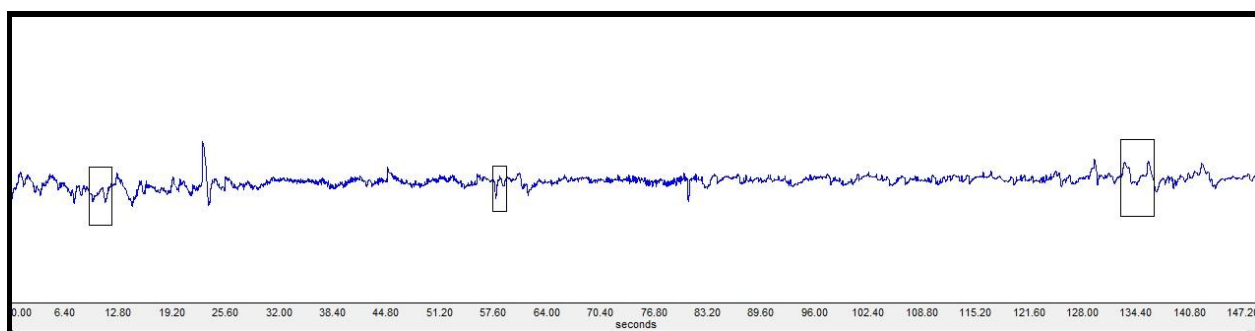
**Figure 16: Electrocardiogram of an Individual Egg Incubated Under Complete Darkness.** EKG's were run on select eggs from the treatment. Eggs were incubated under complete darkness for the entire incubation period. EKG's that showed clear, visible signs of a heart beat were chosen and average beats per minute (BPM) were determined using single beats (one P-P wave or one R-R wave). This was replicated three times on each viable egg, and then the BPM's were average to yield a single average BPM for the egg. Boxes represent the single beats that were selected to determine BPM. Average BPM was determined to be 13.63125.

### *Effects of Varying Wavelengths on Heart Rate*

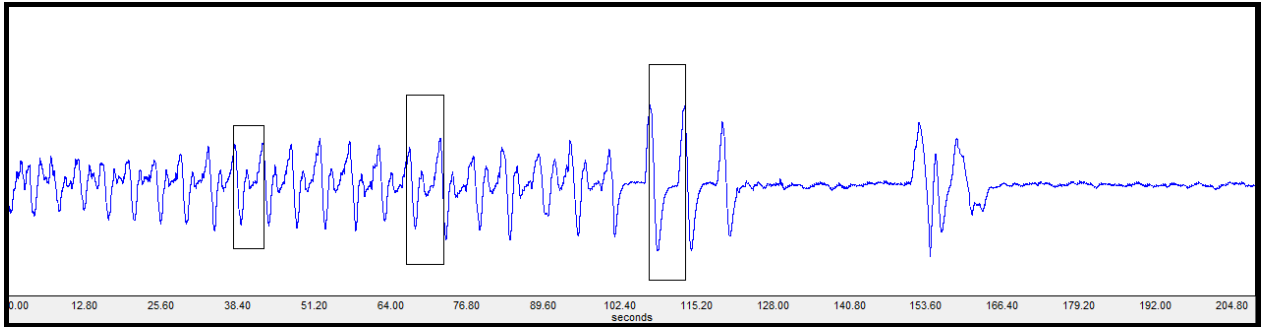
Figure 17, figure 18, figure 19, and figure 20 are from individual eggs incubated under constant red light. The four figures show separate eggs, incubated in the same experimental condition; although, figure 18 and figure 19 shows the heart rate of the same individual egg.

Figure 18 shows the heart rate from an early day, while figure 19 shows the heart rate of the egg

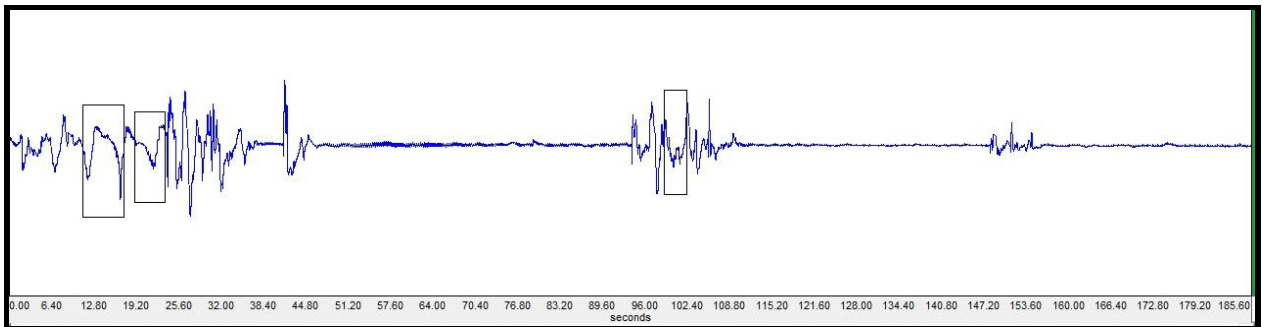
on the last day of incubation. EKG's were run on a max of three minutes, and tended to get shorter throughout the incubation period. Boxes placed on the electrocardiograms represent the single beats that were used to determine the individual heart rate. The triplicates were analyzed and averaged together to give an average BPM for each selected EKG. Average BPM for the selected eggs was 18.80525, 11.18948, 13.73539, and 21.51084, respectfully; yielding an average of 16.32479 BPM



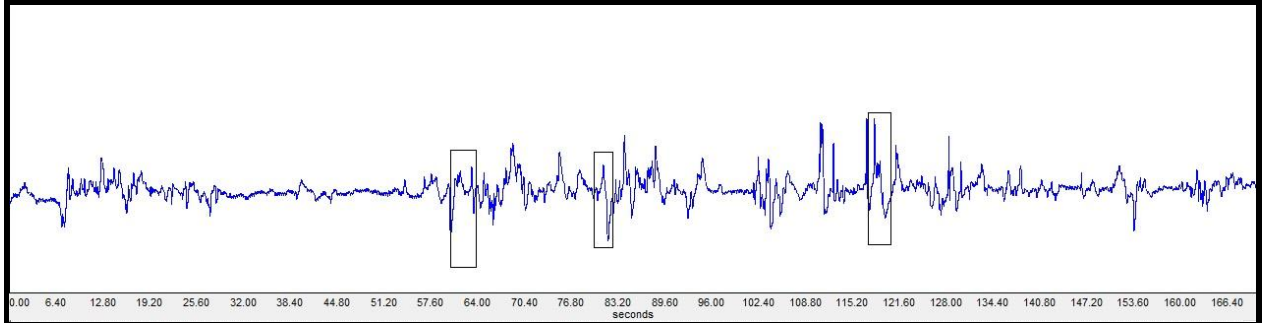
**Figure 17: Electrocardiogram of an Individual Egg Incubated Under Constant Red Light.** EKG's were run on select eggs from the treatment. Eggs were incubated under constant red light for the entire incubation period. EKG's that showed clear, visible signs of a heart beat were chosen and average beats per minute (BPM) were determined using single beats (one P-P wave or one R-R wave). This was replicated three times on each viable egg, and then the BPM's were average to yield a single average BPM for the egg. Boxes represent the single beats that were selected to determine BPM. Average BPM was determined to be 18.80525.



**Figure 18: Electrocardiogram of an Individual Egg Incubated Under Constant Red Light.** EKG's were run on select eggs from the treatment. Eggs were incubated under constant red light for the entire incubation period. EKG's that showed clear, visible signs of a heart beat were chosen and average beats per minute (BPM) were determined using single beats (one P-P wave or one R-R wave). This was replicated three times on each viable egg, and then the BPM's were average to yield a single average BPM for the egg. Boxes represent the single beats that were selected to determine BPM. Average BPM was determined to be 11.18948.

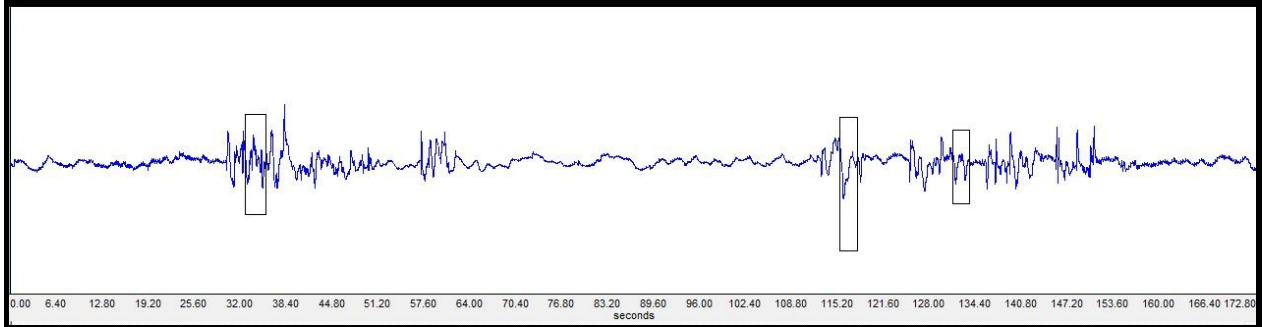


**Figure 19: Electrocardiogram of an Individual Egg Incubated Under Constant Red Light.** EKG's were run on select eggs from the treatment. Eggs were incubated under constant red light for the entire incubation period. EKG's that showed clear, visible signs of a heart beat were chosen and average beats per minute (BPM) were determined using single beats (one P-P wave or one R-R wave). This was replicated three times on each viable egg, and then the BPM's were average to yield a single average BPM for the egg. Boxes represent the single beats that were selected to determine BPM. Average BPM was determined to be 13.73539.

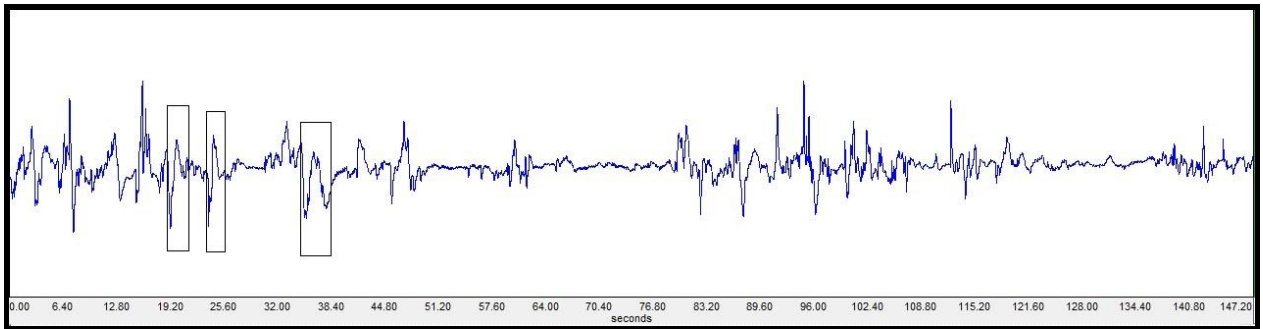


**Figure 20: Electrocardiogram of an Individual Egg Incubated Under Constant Red Light.** EKG's were run on select eggs from the treatment. Eggs were incubated under constant red light for the entire incubation period. EKG's that showed clear, visible signs of a heart beat were chosen and average beats per minute (BPM) were determined using single beats (one P-P wave or one R-R wave). This was replicated three times on each viable egg, and then the BPM's were average to yield a single average BPM for the egg. Boxes represent the single beats that were selected to determine BPM. Average BPM was determined to be 21.51084.

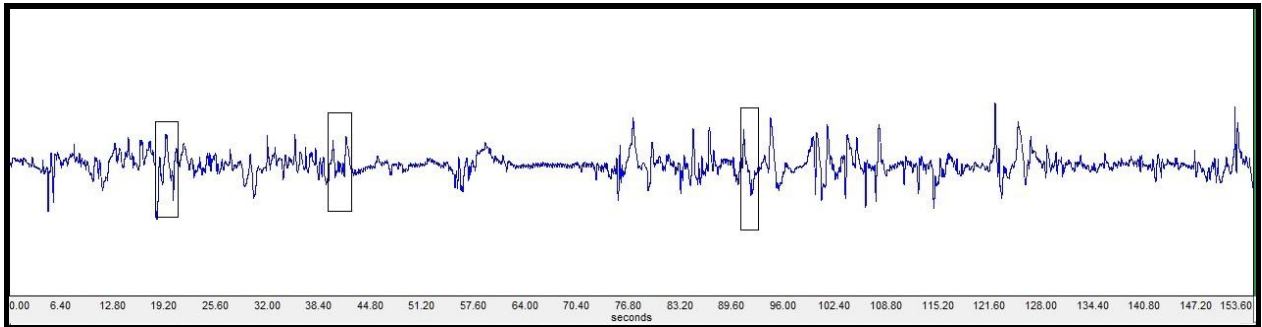
Figure 21, figure 22, figure 23 and figure 24 are from individual eggs incubated under constant blue light. The figures show separate eggs, incubated in the same experimental condition; although, figure 21 and figure 22 shows the heart rate of the same individual egg, and figure 23 and figure 24 shows the heart rate of the same individual egg. Figure 21 shows the heart rate from an early day, while figure 22 shows the heart rate of the egg on the last day of incubation, where the same holds true for figure 23 and figure 24. EKG's were run on a max of three minutes, and tended to get shorter throughout the incubation period. Boxes placed on the electrocardiograms represent the single beats that were used to determine the individual heart rate. The triplicates were analyzed and averaged together to give an average BPM for each selected EKG. Average BPM for the selected eggs was 28.65018, 27.85943, 29.61677, and 32.40682, respectfully; yielding an average of 29.63330 BPM.



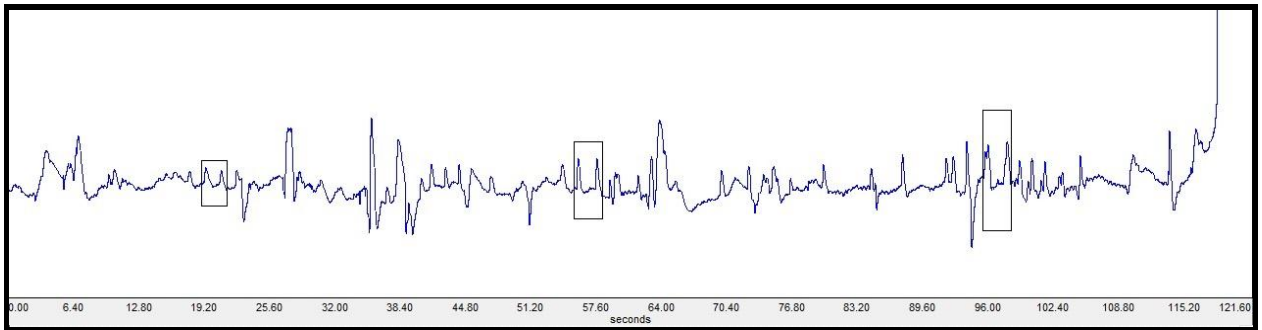
**Figure 21: Electrocardiogram of an Individual Egg Incubated Under Constant Blue Light.** EKG's were run on select eggs from the treatment. Eggs were incubated under constant blue light for the entire incubation period. EKG's that showed clear, visible signs of a heart beat were chosen and average beats per minute (BPM) were determined using single beats (one P-P wave or one R-R wave). This was replicated three times on each viable egg, and then the BPM's were average to yield a single average BPM for the egg. Boxes represent the single beats that were selected to determine BPM. Average BPM was determined to be 28.65018.



**Figure 22: Electrocardiogram of an Individual Egg Incubated Under Constant Blue Light.** EKG's were run on select eggs from the treatment. Eggs were incubated under constant blue light for the entire incubation period. EKG's that showed clear, visible signs of a heart beat were chosen and average beats per minute (BPM) were determined using single beats (one P-P wave or one R-R wave). This was replicated three times on each viable egg, and then the BPM's were average to yield a single average BPM for the egg. Boxes represent the single beats that were selected to determine BPM. Average BPM was determined to be 27.85943.



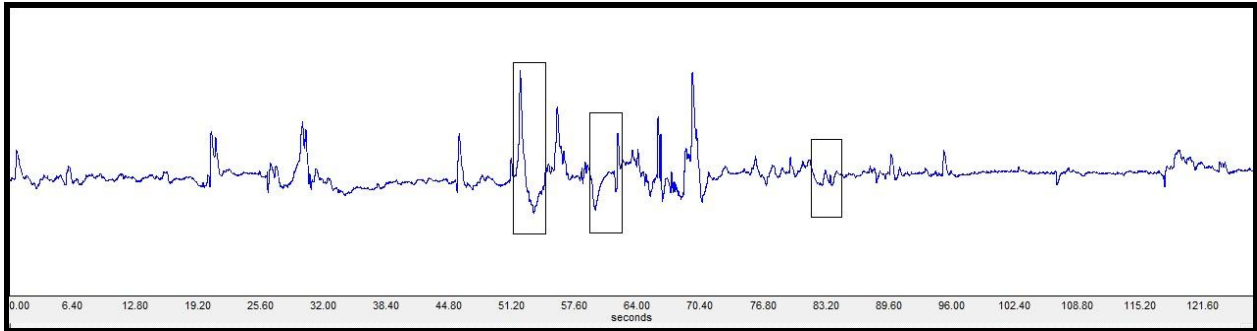
**Figure 23: Electrocardiogram of an Individual Egg Incubated Under Constant Blue Light.** EKG's were run on select eggs from the treatment. Eggs were incubated under constant blue light for the entire incubation period. EKG's that showed clear, visible signs of a heart beat were chosen and average beats per minute (BPM) were determined using single beats (one P-P wave or one R-R wave). This was replicated three times on each viable egg, and then the BPM's were average to yield a single average BPM for the egg. Boxes represent the single beats that were selected to determine BPM. Average BPM was determined to be 29.61677.



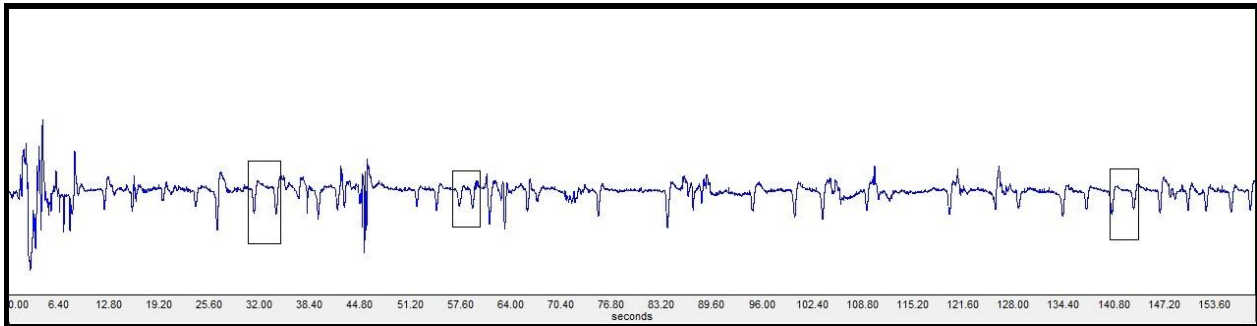
**Figure 24: Electrocardiogram of an Individual Egg Incubated Under Constant Blue Light.** EKG's were run on select eggs from the treatment. Eggs were incubated under constant blue light for the entire incubation period. EKG's that showed clear, visible signs of a heart beat were chosen and average beats per minute (BPM) were determined using single beats (one P-P wave or one R-R wave). This was replicated three times on each viable egg, and then the BPM's were average to yield a single average BPM for the egg. Boxes represent the single beats that were selected to determine BPM. Average BPM was determined to be 32.40682.

Figure 25 and figure 26 are from an individual egg incubated under constant ultraviolet (UV) light. Figure 25 shows the heart rate from an early day, while figure 26 shows the heart rate of the egg on the last day of incubation. EKG's were run on a max of three minutes, and tended to get shorter throughout the incubation period. Boxes placed on the electrocardiograms represent the single beats that were used to determine the individual heart rate. The triplicates

were analyzed and averaged together to give an average BPM for each selected EKG. Average BPM for the selected eggs was 22.93761 and 17.53533, respectively; yielding an average of 20.23647 BPM.



**Figure 25: Electrocardiogram of an Individual Egg Incubated Under Constant Ultraviolet Light.** EKG's were run on select eggs from the treatment. Eggs were incubated under constant ultraviolet (UV) light for the entire incubation period. EKG's that showed clear, visible signs of a heart beat were chosen and average beats per minute (BPM) were determined using single beats (one P-P wave or one R-R wave). This was replicated three times on each viable egg, and then the BPM's were average to yield a single average BPM for the egg. Boxes represent the single beats that were selected to determine BPM. Average BPM was determined to be 22.93761.



**Figure 26: Electrocardiogram of an Individual Egg Incubated Under Constant Ultraviolet Light.** EKG's were run on select eggs from the treatment. Eggs were incubated under constant ultraviolet (UV) light for the entire incubation period. EKG's that showed clear, visible signs of a heart beat were chosen and average beats per minute (BPM) were determined using single beats (one P-P wave or one R-R wave). This was replicated three times on each viable egg, and then the BPM's were average to yield a single average BPM for the egg. Boxes represent the single beats that were selected to determine BPM. Average BPM was determined to be 17.53533.



## CHAPTER FOUR:

### DISCUSSION

Normal development and the growth rate of the avian embryo may be influenced by several environmental factors, notably temperature, atmospheric gases, and humidity (Lauber, 1975). The combined body of evidence, from previous research on domesticated birds and from my experimental evidence, suggests that light can also accelerate embryogenesis within in birds. Embryos showed significantly reduced egg mass as the amount of constant ambient light increased, even when all of the eggs were incubated at the same temperature and humidity. Avian eggs exposed to light throughout the incubation period, develop faster than those exposed to constant darkness. Embryonic metabolic rate is a direct function of egg mass, which has been attributed to an eggshell-conductance limitation of oxygen transport (Pearson *et al*, 1998). Thus, embryonic heart rate is directly related to the embryonic metabolic rate seen within the organism.

Avian eggs exposed to light throughout the incubation period, develop and ultimately hatch earlier than those incubated in complete darkness (Brown, 2004). However, there also appears to be a graded effect depending on the length of illumination. Thereby, eggs incubated in complete darkness would take longer to fully develop within the incubation period, whereas, eggs incubated in constant light would take the least amount of time. Eggs incubated under the temperate light cycle (18:6) showed an increased time of hatchability. Eggs incubated under this light regime were comparable to the constant light cycle control treatment. Eggs that were treated using the equatorial light regime (12L:12D) were more intermediate in development, and

therefore, had a reduced metabolic rate when compared to the all light control. However, all treatments did show signs of development throughout the incubation period. All weights tended to decrease in weight over the length of incubation, however, the rate at which they decreased varied across light regimes.

Changes in heart rate during growth reflect the changing metabolic requirements of the developing embryo (Pearson *et al*, 1998). The general pattern of heart rate typically involves an asymptotic increase with embryonic development early in incubation to a plateau of about 300 beats/min, at which point a cardiogenic signal is first detectable around day 7 (Brown, 2004, Pearson *et al*, 1998). However, in this study, the heart rate for all embryos did not follow this generally observed pattern. Heart rates were not statistically able to be calculated on each individual selected egg, leaving some of the data up to extrapolation and interpretation. In general, however, there is considerable variation in the rate of heart beat between individual embryos (Romanoff, 2005).

Heart rate and metabolic rate in the avian embryo are assumed to be closely coupled, such that a change will lead to a change in heart rate, while heart rate supports the metabolic rate by being a large contributor toward supplying metabolic demands to the embryo (Brown, 2004). Heart rate tends to develop a rhythm due to the light-induced zeitgeber, as opposed to any other external factor. Embryonic metabolic rate at the pre-internally pipped stage is a function of egg mass (Pearson *et al*, 1998).

Photoperiod, affects embryonic growth and development (Cooper *et al*, 2011). The rate and mechanism of photoacceleration seen in birds varies with embryonic development. Light stimulation produces the fastest rates of embryonic development during incubation (Cooper *et al*, 2011). Once the primary components of the avian circadian rhythm are matured, light is able to

stimulate embryonic growth and development. Light appears to stimulate mitosis in the neural crest mesoderm during the first couple days of chicken embryo development, accelerating the closure of the neural tube (Cooper *et al*, 2011). This is consistent in that high light intensity increases embryonic cell proliferation (Cooper *et al*, 2011). Light is also able to penetrate to the cellular level, directly activating cytochromes within the inner mitochondrial electron transport chain, stimulating cellular metabolism (Cooper *et al*, 2011). Cyclic AMP production is also regulated by light, leading to the influence of gene expression within developing embryos. After development of the pineal gland, the entrainment of the avian embryo to photoperiod is mediated by the production of melatonin (Cooper *et al*, 2011). Light reduces the production of melatonin, while darkness increases the production; showing the effects of the fluctuating photoperiod on the internal clock of birds. The effective biological relevance of sunlight on embryonic development in the wild may depend on (i) the relative importance of circadian rhythms compared to additive effects, (ii) interactions with other influential features of the embryonic environment, such as temperature, habitat, and parental behavior that influence how photoperiod translates into the amount and quality of light received by embryos, and (iii) the importance of phylogenetic constraints and local adaptations (Cooper *et al*, 2011).

As indicated by both experiments, light used as a zeitgeber can induce embryonic development, yet varies with light intensity and wavelength. In order to examine the effect of light on avian development, the ability of quail (*Coturnix coturnix*) to grow under various light regimes was tested using an all light control, a temperate (18L:6D) light cycle, an equatorial (12L:12D), an all dark control; as well as various wavelength cycles (red light, blue light, ultraviolet light). Eggs developed most strongly under the regimes that contained the most light. This can be seen in both egg mass, as well as average observed heart rate. The temperate control

group showed the most reduction in egg mass, when compared to the controls. Due to previous research mentioned by Cooper, *et al* this was to be expected and was my original hypothesis (Cooper *et al*, 2011). Eggs showed the sharpest decrease in mass during the incubation period. Development was assumed to be accelerated due to the quantity of light. The all light regime and temperate treated eggs showed the strongest signs of development throughout the incubation period; whereas, the equatorial and dark cycles were more comparable through the data. As the amount of ambient light started to decrease across treatments, egg mass was retarded, thus, slowing down developmental processes.

The same comparable data can be seen through analysis of the electrocardiogram. The increase in artificial light increased average BPM within the tested eggs. All four light treatments contained two eggs for EKG analysis, so statistically comparable data was able to be drawn. It is to be noted that the embryonic heart rates are not statistically accurate when compared to the average BPM seen within a previous study conducted by Pearson *et al*. Not enough viable EKG's from the eggs were able to be used when determining heart rate within the embryos. Invasive techniques for determining heart rate may truly be detrimental to the developing embryo within, leaving non-invasive techniques with more accurate data. However, it is to be noted that the data still follows the same generalized pattern seen within egg mass. Treatments that contained the most artificial light produced the highest average BPM within the eggs. The temperate light regime produced the highest heart rate; however, it was hypothesized that the 24-hour control group should have produced the highest heart rate. Although, the two are relatively close, and much higher than the two treatments containing less light, and more darkness; this allows for the conclusion that light can also be used as an external cue for development. Results

found within this study showed that embryonic development is accelerated by light, when temperature and environmental gas pressures are held constant.

Light spectra also has an effect on avian growth. Rozenboim, *et al* demonstrated that birds raised under monochromatic blue light gained more weight than those raised under red light (Rozenboim *et al*, 1999, Rozenboim *et al*, 2004). Blue light advances early chick-embryo growth (Rozenboim *et al*, 1999). Eggshells typically act as a protectant from harmful radiation, however, short wavelengths (i.e. blue light) is able to penetrate through the shell and be absorbed by embryonic cells (Rozenboim *et al*, 1999). Longer wavelengths (i.e. red light) may be unable to pass due to the pigmentation of the shell. With the harmful rays being deflected away by the shell, shorter, more potent rays may still be able to be absorbed, allowing for ultraviolet (UV) light to actually stimulate growth within the cells. Typically, UV light is viewed as a mutagen, negatively affecting growth and development within organism. However, with the shell acting as a protectant, less harmful rays may be able to penetrate the cells stimulating growth.

The wavelength dependence of the photoperiodic mechanism is shown by the data from experiment 2. As hypothesized, blue light affected the growth within the embryos the most. Eggs showed the most reduction in mass under the blue light incubation. The shorter wavelength was able to penetrate the shell and advance embryonic growth as previously proposed by Rozenboim *et al*. With respect to the longer wavelength, the red light was still able to augment development within the embryos; however, weight reduction was not increased to the same degree as seen within the all light control, or even that seen in eggs incubated under blue light. These findings were consistent with the original hypothesis of this study. However, eggs treated under ultraviolet light showed intermediate mass reduction throughout the incubation period. It was still expected that egg mass reduction under UV light would be statistically less than the

reduction seen in eggs treated with blue light. UV light acting to stimulate growth faster than that of red light does warrant further research. This is interesting that UV light which is typically regarded as detrimental to embryogenesis is indicated in this experiment to augment embryonic growth.

Analysis of the electrocardiogram for experiment 2 yields the equivalent data seen in egg weight. It is important to note again that the embryonic heart rates obtained in this experiment are not statistically accurate when compared to the average BPM seen within a previous study by Pearson *et al* research study (Pearson *et al*, 1999). Not enough viable EKG's from the eggs were able to be used when determining heart rate within the embryos. Invasive techniques for determining heart rate may truly be detrimental to the developing embryo within, leaving non-invasive techniques with more accurate data. However, it is to be noted that the data still follows the same generalized pattern seen within egg mass. Blue light incubated eggs contained the highest average BPM within the developing eggs. As proposed by Rozenboim *et al.*, blue light stimulates the growth of embryonic cells, thus, an increase in metabolic rate is required; thereby, increasing the average BPM within the embryo (Rozenboim *et al*, 1999, Rozenboim *et al*, 2004). Red light produced the lowest average BPM within the eggs, showing similar results of the egg weight. The longer wavelength is unable to penetrate the pigments of the eggshell, retarded the embryonic growth. Average BPM of eggs reared under red light is statistically similar to the average BPM of eggs in complete darkness. A large portion of the light rays may be actually getting deflected away by the shell that the microenvironment within the egg is similar to that of complete darkness.

Results found within this study showed that embryonic development can be accelerated by light spectra, when temperature and environmental gas pressures are held constant. Light was

found to directly influence the rate of development within the embryo. The luminance of light, as well, as color of light are important factors that will continue to influence avian productivity.

Artificial illumination will continue to significantly influence avian growth in the modern poultry incubation. Therefore, further research on how light increases avian production can be used to further increase the yield within the poultry industry.

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## Education

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## Honors and Awards

- 2014 School of Science Student Marshal
- Penn State Behrend Men's Scholar Athlete of the Year (2014 )
- 2012 Capital One Academic All-District Men's Soccer Team
- 2013 National Soccer Coaches Association of America (NSCAA) College Division Scholar All-East Region team

## Association Memberships/Activities

- Penn State Schreyer Honors College
- Penn State Behrend Men's Soccer Team
- Beta Beta Beta Honors Society
- Lambda Sigma Honors Society
- Penn State Behrend Honors College
- TOPS Soccer Program

## Research Experience

- Effects of Knocking Down MLO Gene Expression in *Vitis vinifera* on Regulation of Susceptibility to Powdery Mildew
- Poster Presentation: Effects of Knocking Down MLO Gene Expression in *Vitis vinifera* on Regulation of Susceptibility to Powdery Mildew
- Photoacceleration of Embryonic Development of Quail (*Coturnix coturnix*) Under Different Stimuli of Light

## Professional Presentations

- Poster Presentation: Effects of Knocking Down MLO Gene Expression in *Vitis vinifera* on Regulation of Susceptibility to Powdery Mildew (Sigma Xi Research Conference – Spring 2013, Penn State Behrend, Erie, PA)