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EVALUATION OF HANDLING EQUIPMENT SOUND PRESSURE LEVELS AS STRESSORS IN BEEF CATTLE

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THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in
Biosystems and Agricultural Engineering
in the College of Engineering at the University of Kentucky

By

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ABSTRACT OF THESIS

EVALUATION OF HANDLING EQUIPMENT SOUND PRESSURE LEVELS AS STRESSORS IN BEEF CATTLE

Sound pressure level (SPL) is known to cause stress in cattle but is often overlooked as a potential source of fear for cattle when designing handling equipment. Current literature does not offer guidelines for the design of equipment with regard to SPL. It is, however, recommended that handling equipment should be designed to minimize the SPL during handling. The purpose of this experiment was to measure stress levels in a group of cattle which were subjected to a series of varying sounds in order to determine a design threshold limit for handling equipment. Treatments included two frequencies, 1 kHz and 8 kHz, and three intensities, 40, 80, and 120dB. These treatments were assigned to the cattle using a completely randomized two by three factorial design replicated three times for a total of 18 animals being tested. A computer generated noise at each level was played back to the animals once a week for 6 weeks. Stress levels were measured using both physiological (heart rate and eye temperature) and physical (sudden movement) measures. Experiments yielded mixed results and did not prove that any of the sound pressure levels tested had any great effect on the stress level of the cattle.

KEYWORDS: Beef Cattle, Sound Pressure, Stress, Handling Facilities, Noise

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1/14/13

EVALUATION OF HANDLING EQUIPMENT SOUND PRESSURE LEVELS AS STRESSORS IN BEEF CATTLE

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To my parents and family, who have always believed in me and only expected the best.

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CHAPTER 1 Introduction

Reducing livestock stress during handling will provide advantages of increasing productivity and improving meat quality (Grandin, 1998). Previous laboratory work has indicated that cattle agitated and excited in the squeeze chute have significantly lower weight gains, tougher meat, and more borderline dark cutters (Voisinet, 1997). Dark cutters is a meat quality defect characterized by elevated muscle pH (>6); high water-holding capacity; dry, firm, and "sticky" lean; and a dark-red to almost black lean color (Apple, 2005). It has been found that dark cutters cost the U.S. beef industry $132-$170 million annually (Smith et al., 1995).

Sound pressure level (SPL) is known to cause stress in cattle but is often overlooked as a potential source of fear for cattle when designing handling equipment. Current literature does not offer guidelines for the design of equipment with regard to SPL. It is, however, recommended that handling equipment should be designed to minimize the SPL during handling (Grandin, 1998).

1.1 Objectives

1.1.1 Goal

The goal of this study was to measure stress levels in a group of cattle that were subjected to a series of varying sounds in order to determine design threshold limits for handling equipment.

1.1.2 Specific Objectives

1) Determine a range of frequencies and intensities produced during normal operation of a squeeze chute used by the typical Kentucky beef cattle producer.
2) Evaluate any correlation between stress level in cattle and sound pressure level.
3) Provide a recommended range of SPL to be used by manufacturers during the design of handling equipment.

CHAPTER 2 Literature Review

2.1 Stress

Successful completion of this project will require a fundamental understanding of animal stress. The term “stress” has no clear definition for use in animal husbandry. Moberg (2000) defines stress as a biological response elicited when an individual perceives a threat to its homeostasis. The threat is the “stressor”. Borell (2001) describes stress as a broad term that implies a threat to which the body needs to adjust. There are two different types of animal stress; psychological and physical. Psychological stress may be initiated by restraint, handling, or novelty and physical stress is a result of hunger, thirst, fatigue, injury, or thermal extremes (Grandin, 1997).

2.2 Sound as a Stress Risor

The hearing range of cattle at 60 dB extends from 23 Hz to 35 kHz (Heffner et al., 1983). Ames (1974) indicates that the auditory sensitivity of cattle peaks at 8000 Hz. The human ear is most sensitive between 1000 and 3000 Hz. Figure 2-1 shows the audiogram of four different mammals, cow, human, horse, and elephant. This figure shows that cattle have more sensitive hearing than humans, and noises that are a whisper to humans are quite audible to cattle. Besides an elephant, cattle are known to possess better low-frequency hearing than any other mammal (Heffner et al, 1982). This increased sensitivity makes noises such as equipment rattling, metal on metal clanging, and noise created by humans, prime areas for potential auditory stressors.

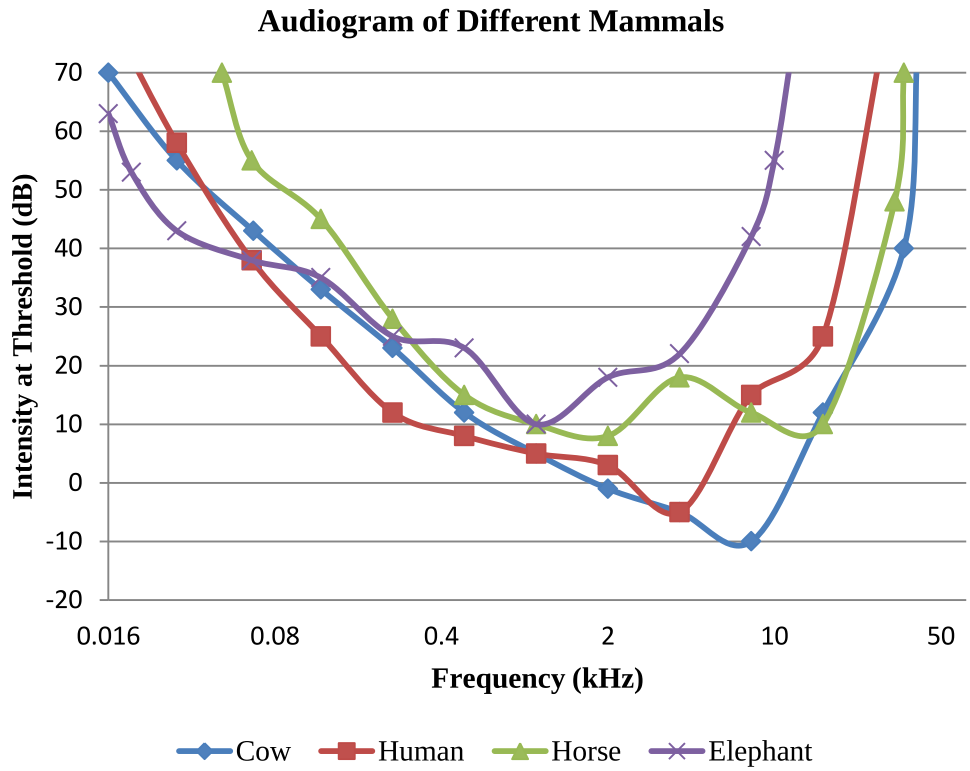

Figure 2-1 Audiogram of different mammals
(Heffner et al, 1982, 1983, 1983b)

A group of researchers in Canada performed two different experiments to look at beef cattle response to noise (Waynert et al., 1998). The researchers in this experiment used the heart rate as their physiological measurement and then the animal's movement as the behavioral measurement of the animal's welfare. The experiment was broken down into two different trials; effects of noise and metal clanging vs. shouting. Each trial was then broken into two groups for the testing. During the first trial one group of heifers was subjected to noise while being handled and the second group was handled while being kept in silence. The noise treatment was a combination of human voices and metal clanging. The animals subjected to the noise treatment exhibited higher heart rate compared to the animals kept in silence. The noise treatment group also had a larger change in the amount of movement. Next, the researchers compared the response of the animals when exposed to metal clanging vs. people shouting. This time the sound of the people shouting caused the animal's heart rate and movement to increase more than the metal clanging. Although this research proved that the animals had more response to the people shouting, it does not disprove the need to reduce the noise level of the metal clanging together. The authors acknowledged that reduction of both forms of noise during handling is needed to help reduce the level of fear in beef cattle.

Unexpected loud or novel noises can be highly stressful to livestock. Sheep exposed to exploding firecrackers or noises in a slaughter plant were found to have an increased thyroid hormone level and elevated cortisol (Falconer et al. 1964, Pearson et al, 1977). During a personal interview at the USDA Experiment Station, T. Grandin (1989) was told that it had been found that the loud clanging bell from an outdoor telephone will raise a calf's heart rate 50-70 beats per minute. Lainer (2000) found that the stimuli that were most effective for eliciting a startle response in cattle were intermittent, high-pitched sounds and sudden movements. Talling et al (1996) also found that high-pitched sounds have a greater effect on an animal's heart rate than low-pitched sounds.

Domestic pigs, like cattle, are most sensitive to noise at 8000 Hz (Heffner et al, 1990). Talling et al (1996) found that the higher frequency tone 8000 Hz resulted in a higher heart rate than a 500 Hz tone in pigs. In a second study, Talling compared the increase of heart rate in pigs exposed to four different recordings. The recordings were as follows:

1. Farm: Indoor, partially slatted, ventilated, fattening yard containing 30 pigs. Sound content: pigs grunting, occasionally squealing, ventilation fan. SPL: 80dB
2. Transporter: Front of the top deck of a transporter travelling at 55mph holding 25x20kg piglets in pen. Sound content: piglets squealing, gates banging, engine sound, tire sound. SPL:83dB
3. Abattoir: Pre-stun pen, water spray, 70kg pigs passing through pen, manual shackle line in stun pen. Sound content: pigs squealing continuously, men shouting, water spray, shackle line conveyors, gates banging. SPL: 84.
4. White noise: All frequencies from 100 Hz to 10,000 Hz at approximately the same SPL. SPL:89dB

The transporter recording resulted in the greatest increase in heart rate (14.2bpm) followed by the white noise (11 bpm) and the farm recording (7.1 bpm). The increase in heart rate due to the abattoir recording was not significantly different from the control. Talling concluded that these two studies suggest that novel sound is an arousing stimulus that initially activates the animals' defense mechanisms.

Nosal and Gygaz (2004) performed a study looking at the effect of noise and vibration as a stress factor in milking. One of the farms examined reported the noise level was reduced from 79 to 55 dB. Results showed a reduction of somatic cell count from 450,000 to 120,000. These reduce cell numbers were also accompanied by the increase of milk production from 7400 to 8100 liters (Nosal and Gygaz, 2004).

2.3 Measurement of Stress

A combination of behavioral and physiological measurements must be made in order to properly assess an animal's welfare during handling. Behavioral measures are observations of the animal doing any of the following: attempting to escape, vocalization, kicking or struggling. Physiological measurements of stress include the level of cortisol, beta endorphins, or the animal's heart rate. (Grandin, 1997)

2.3.1 Hormones

Cortisol is a mammalian hormone that is released routinely. The release of cortisol is often increased in response to stress (Bristow, 2006). Cortisol is a useful indicator of short-term stress from handling or husbandry procedures such as castration (Lay et al, 1992). However cortisol is a time dependent measure that takes 10 to 20 minutes to reach peak values (Lay et al, 1992). Glucocorticoids, such as cortisol, also have a circadian rhythm in many species. This rhythmicity and episodic secretion demands frequent sampling in order to accurately detect a stress induced response. The concentration of cortisol in an animal's body can be sampled via blood, urine, feces, saliva, or milk. Some researchers have developed remote blood sampling devices to help reduce the stress on the animal during sample collection. One of the major advantages of fecal samples is that they can be easily collected without stressing the animals (Mostl, 2002).

Grandin (1997) reviewed multiple experiments and tentatively concluded that a mean value of >70ng/mL in steers or cows would possibly be an indicator of rough handling or poor equipment, and low values closest to the baseline values would indicate that a procedure was either low stress or was very quick. Quick procedures would be completed before cortisol levels could increase.

2.3.2 Heart Rate

Heart rate (HR) is another way to measure cardiac activity. Borell et al (2007) indicates HR in cattle can be used to measure stress from physical, pathological and emotional origins. The calves wear the heart rate monitors for an hour to facilitate to become accustom to the equipment (Borell et al, 2007) and get proper HR readings.

2.3.3 Infrared Thermography

The hypothalamic-pituitary-adrenal (HPA) axis is activated when an animal becomes stressed and there is a change in metabolic heat production by the animal. Infrared thermography (IRT) is the measurement of radiated electromagnetic energy. Electromagnetic radiation is described as a stream of photons travelling in a wave-like pattern and moving at the speed of light. The photons with the highest energy correspond to the shortest wavelengths. Broad range infrared radiation wavelengths (3-12 μm) in the electromagnetic spectrum are longer than visible light. In animals, 40 to 60% of heat loss is within this range (Kleiber 1975). The heat production is a result of an increase in catecholamine and cortisol levels as well as blood flow (Schaefer et al, 2002). The change in heat loss can be detected using an infrared camera to record real-time thermal images of the animal. Small changes in temperature may result in substantial amounts of emitted photons that can be detected very accurately using IRT (Stewart et al 2005).

There are some things that have to be considered in order to successfully collect data using IRT. Images must be collected out of direct sunlight and wind drafts, and hair coats should be free of dirt, moisture or foreign material. Dirt and other foreign material on the animal alter the emissivity and thermal conductivity of the hair coat. Excess moisture increases local heat loss to the environment (Palmer, 1981).

2.3.3.1 Eye Temperature

Stewart et al (2007b) conducted experiments which showed the maximum temperature within the area of the medial posterior palpebral border of the lower eyelid and the lacrimal caruncle was recorded on average every 38 sec immediately into an excel spreadsheet throughout the entire 40 min sampling period.

Heart rate increases and blood flow is redirected away from the extremities to organs and musculature (vasoconstriction) during the ‘fight or flight’ reaction. Stewart et al (2007b) found a rapid drop in eye temperature after disbudding of calves without local anesthetic using IRT.

Stewart et al (2007b) conducted an experiment to determine if eye temperature, measured by IRT, could non-invasively detect responses of cattle to various handling procedures. Six heifers were randomly assigned to two groups and received two treatments in a crossover design. One treatment, hitting, consisted of three brief slaps on the rump with a 1m length of plastic tubing. The second treatment, startling, consisted of two brief sudden shakes of a plastic bag in front of the animal’s head. Infrared images of the eye region were collected using an infrared camera placed a consistent distance (.5 m) and angle (90°) from the left side of the animal. The area of analysis was restricted to the medial posterior palpebral border of the lower eyelid and the lacrimal caruncle. The results showed that the eye temperature dropped rapidly between 20 to 40 second by $0.23℃ \pm 0.08$ after hitting. The eye temperature dropped during the same time frame $0.32℃ \pm 0.05$ after startling. Eye temperature returned to baseline levels between 60 and 80 seconds following hitting and between 100 and 120 seconds following startling.

2.4 Behavior

2.4.1 Ethogram

An ethogram is a catalog of typical behaviors exhibited by a certain species. An ethogram provides a benchmark against which one can measure behavioral deviations and then evaluate recommendations to determine if they are effective in moving the behavior back toward normal. Behavior is defined as an aspect of the animal’s phenotype that involves the presence or absence of definable motor activities, vocalizations and odor production while conducting its daily affairs of self-maintenance and social interaction.

Units of behavior are called Modal Action Patterns (MAP) and are often measured by the frequency and duration of the behavior (Banks, 1982).

There are two different categories of behaviors that need to be considered when developing a strategy for an ethogram; those displayed by solitary animals and those associated with animals maintained in groups. Animals in solitary can be observed performing behaviors such as feeding, drinking, sleeping, self-grooming, pacing, chewing on cage, etc. Animals that are in a group can be observed with the following behaviors: sexual, aggressive, parental, spacing, mutual grooming, play, etc (Banks, 1982).

There are four different scales of measurement of behavioral phenomena: nominal, ordinal, interval, and ratio. The four scales as described by Banks (1982) are summarized in Table 2-1.

Table 2-1 The Four Scales used to measure behavior patterns

Scale	Measurement requirement	Behavior Patterns
Nominal	Mutually exclusive and totally inclusive categories	Presence or absence of aggressiveness, emotionality, etc.
Ordinal	A unidimensional scale	Peck Order, maternal behavior
Interval	A unidimensional scale possessing additivity	Intelligence learning
Ratio	A unidimensional scale possessing additivity and absolute zero	Psychophysical scales of loudness, brightness, and pitch

Finally, after the measurement scale and parameters have been decided, the observer has to decide which observational strategy to use. The five main observational strategies are outlined in Table 2-2 (Banks, 1982).

Table 2-2 Observational sampling strategies

Sampling Method	State or event sampling	Recommended Uses
Ad Libitum	Either	Primarily of heuristic value; suggestive; recording of rare but significant events
Focal-animal	Either	Sequential constraints; percentage of time; rates; durations; nearest neighbor relationships
All occurrences of some behaviors	Usually event	Synchrony; rates
Sequence	Either	Sequential Constraints
Instantaneous and scan	State	Percentage of time; synchrony; subgroups

Stewart et al (2007b) used an ethogram as a third measurement of stress in his experiment comparing the eye temperature and heart rate variability of calves disbudded with or without local anesthetic. The animals are videotaped during the procedure and then the recordings are analyzed later for the behavioral study. Table 2-3 described the behavior he recorded continuously during his sampling period.

Table 2-3 Description of each behavior recorded continuously during the 40 min sampling period (Stewart et al, 2007b)

Behavior	Description
Rear	One or both front legs are raised off the ground in a forward pawing action
Leg Lift	Any food raised off the ground and then replaced, often in a rapid movement (within 2 seconds)
Lunge	Both back legs leap forward or backwards together and land simultaneously
Crouch	Rump lowers to the ground, in a crouching motion, without the calf falling to the ground (recorded when the top of the tail reaches the point of the escutcheon or lower)
Fall	The calf collapses to the ground onto both knees and/or hocks
Slip	Hind leg is extended backward or stretched forward as it slides along the floor
Vocalize	Any Audible noise made by the calf.

2.4.2 Movement

Stookey et al (1994) developed a movement measuring device (MMD) to measure the movement of cattle during restraint. Load cells were attached to the squeeze chute and then connected to the MMD. Analog changes in voltage that occurred as the animal moved into the squeeze chute were sampled at a rate of 122 times/sec for one minute. The MMD recorded a peak when a trend of increasing or decreasing voltages was reversed. The number of peaks then reported on a LCD display. The number of peaks was indicative of the amount of movement made by the animal during the test. The number of peaks increased or decreased depending on how still the animal was during the test.

CHAPTER 3 Methods and Materials

3.1 Preliminary Sound testing

Sound testing was completed using the Tarter Cattlemaster chute in order to determine sound pressure levels that cattle currently experience during handling. The chute was set up in a laboratory setting for the sound testing. Five areas of the chute were deemed to be problem areas to be included in the sound testing. These areas produced the loudest sounds during use based on human observation. The following parts were tested during sound testing:

- Head Gate
- Tailgate
- Side access doors
- Palpation door
- Squeezing mechanism

A full scale 2D model of a cow was placed inside the chute during testing. The cow was designed using Dimensions of Livestock and Poultry ASAE Standard D321.2 (2011). The measurement microphone was placed at four feet from the ground so that it was located at the height of a cow’s ears. The microphone was placed at the front of the chute during testing to record the sounds in the location that the cattle would hear the sounds while in the chute. Figure 3-1 shows the Tarter Cattlemaster Chute during testing with the 2D cow and the measurement microphone.

Figure 3-1 The chute set up for sound testing

The measurement microphone was attached to a computer via universal serial bus (USB) interface. The sound frequency (Hz) and peak sound pressure level (dB) were measured using TrueRTA™ (TrueRTA, 2011) software. Each problem area of the chute was operated during sound testing as it would have been during typical operation. Five sound measurements were taken for each of the five problem areas and then the average was computed.

3.1.1 The Tarter Cattlemaster Chute

Figure 3-2 Tarter Cattlemaster Chute (Tarter 2011)

Squeeze chutes are not a daily use item for a cattle producer but it is still a very key piece of equipment for their operation. The squeeze chute is used by producers to restrain cattle during various veterinary procedures. The Tarter Cattlemaster Chute (Figure 3-2) is a $3,600 investment that is readily available and representative of the handling equipment used by an average sized producer in Kentucky (Tarter 2011).

3.1.2 Hydraulic Squeeze Chute

The hydraulic squeeze chute from Company B was used during testing. It is widely viewed as the industry leader for stress reduction during handling and is marketed to have a noise reduction system applied to over 130 contact points. The very basic model of the hydraulic squeeze chute would be approximately an $18,000 investment.

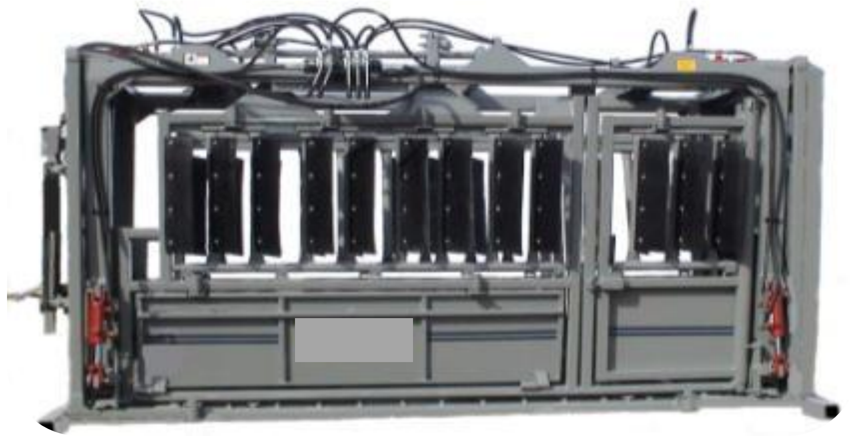

Figure 3-3 Example of hydraulic chute with noise reduction

3.1.3 Measurement Microphone

Sounds levels were measured using a Dayton Audio EMM-6 Measurement Microphone (Dayton Audio). This precision condenser microphone is designed for measurement and critical recording applications. The frequency response of the microphone is 18 Hz – 20 kHz.

Figure 3-4 The EMM-6 Measurement Microphone

The EMM-6 is a relatively inexpensive measurement microphone with a cost of $50. Other microphones were explored that had a broader frequency response but they were more expensive. Table 3-1 below outlines the cost of potential microphones that could have been used for the sound testing. The Dayton Emm-6 is the cheapest option however it does not reach the 35 kHz max of the cattle hearing range. The costs presented in Figure 3-5 show that the microphone from company C exceeds the cattle's upper hearing limit but comes at a cost of $1094.50. The slight increase in the high frequency limit for microphone A and B was not found to outweigh the increase in cost.

Table 3-1 Comparison of Microphones options for Sound Testing

Microphone	Low Frequency Limit	High Frequency Limit	Cost	Cost Increase*
Dayton EMM-6	18 Hz	20 kHz	$48.26	
A	9 Hz	23 kHz	$459	$410.74
B	9 Hz	27 kHz	$649	$600.74
C	4Hz	80 kHz	$1094.50	$1045.24

*** Note: Comparison based on the Dayton EMM-6**

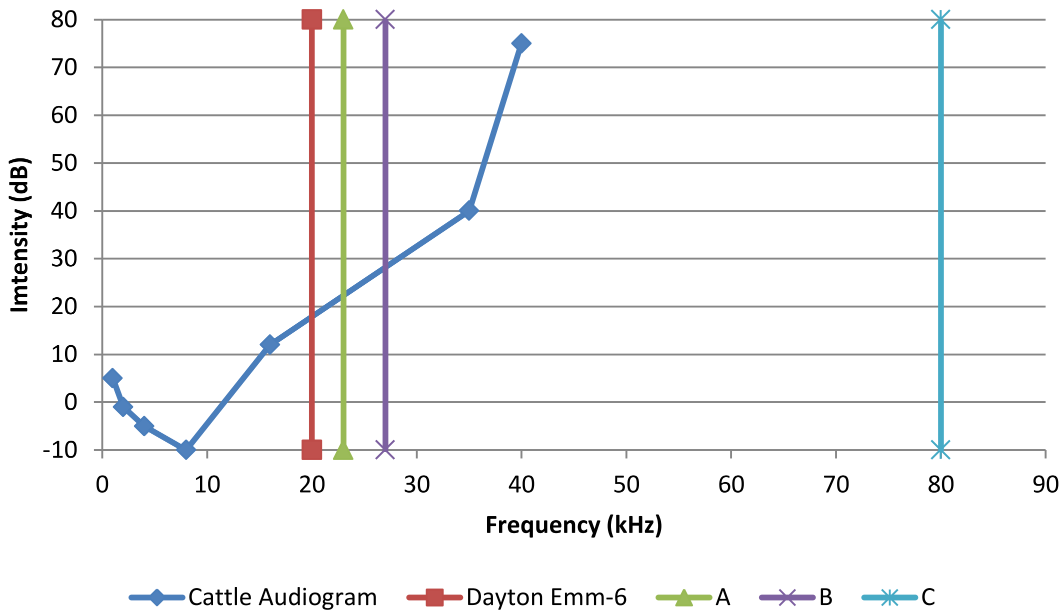

Figure 3-5 Comparison of the upper cutoff limit of the microphones.

3.1.4 *TrueRTA™ Software*

TrueRTA™ (TrueRTA, 2011) is real time audio spectrum analyzer software that was used during the initial sound testing. The combination of the software and the measurement microphone was used to determine the peak sound pressure level (dB) and frequency (Hz) of the sound. The TrueRTA™ software also includes a sound generator used to produce the noises for the stress testing. This ensures that the consistent treatments are applied to during each experiment.

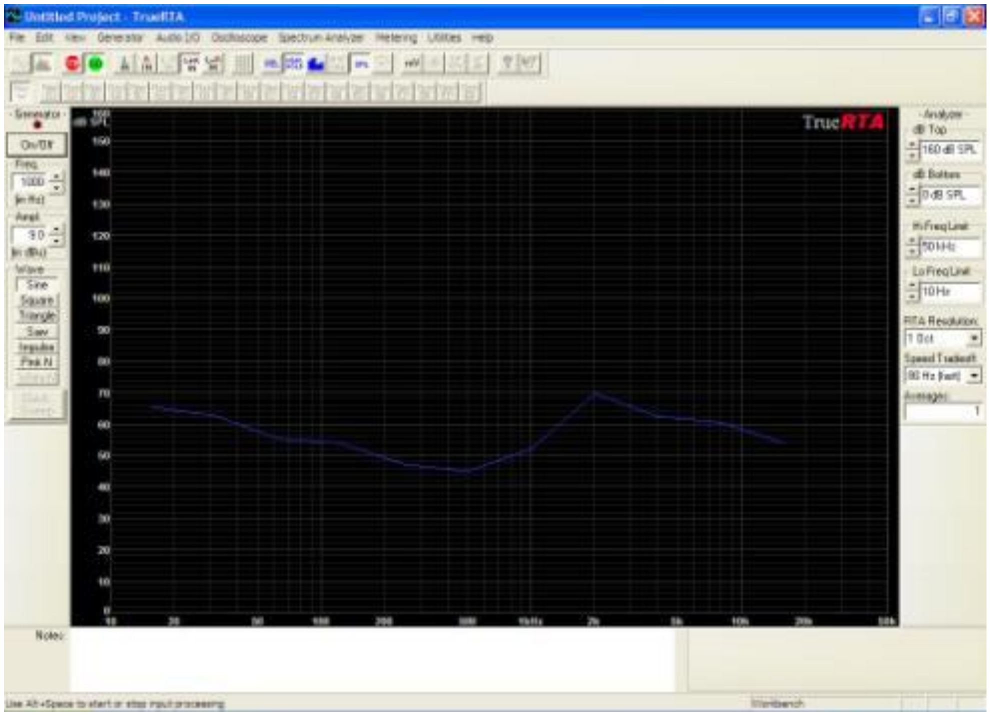

Figure 3-6 Example of the Spectrum Analyzer Output in TrueRTA

3.2 Stress Testing

The stress testing on live animals was completed at the University of Kentucky C.Oran Little Research Farm. All protocols for stress testing were approved by the Institutional Animal Care and Use Committee (IACUC). Stress responses were determined from measurements of heart rate (HR) by remote telemetry, eye temperature by infrared thermography, and sudden movement by digital scales. Heart rate and eye temperature were measured to determine a physiological response to the sounds and movement was measured to indicate a behavioral response. The stress measurements from all methods were compared using the time stamps on the data. Initial sound testing data on the Tarter chute were used to determine that six treatments would be used during testing. Treatments were given to a group of 18 cross bred steers.

3.2.1 Testing Procedure

The testing was carried out once a week in an effort to reduce the effect on the animals' acclimatization to the experiment and the handling facilities. Animals not receiving treatments were held in a staging area 26.8 meters (88 ft.) from the experiment area. They were then coaxed up the alleyway into the chute one at a time to receive their predetermined treatment.

Measurements were taken for three different time periods during the experiment: pretreatment (P1), during treatment (P2), and post treatment (P3). To detect if the treatments resulted in a change of the heart rate, eye temperature and movement, data was collected for one minute prior to the treatment (P1). Subsequently, the animals were subjected to the predetermined treatment level. The sounds were played from a speaker placed 2.5 meters (8.2ft) at an angle of 70° in front of the chute at ear level (Figure 3-7). The heart rate was recorded for a minute following the treatment and is considered the during-treatment measurement (P2). Measurements were also taken for a minute following the during-treatment period (P3) to determine if there was a change in the stress response.

Figure 3-7 Testing set up with infrared camera and speaker

3.2.1.1 Treatments

The six treatments were chosen to cover the range of sound levels produced by the Tarter chute. The frequency levels used for the experiment were the only two frequencies identified from the Tarter chute during sound testing. Table 3-2 shows the letters assigned to the six treatments to be used during the design of the Latin square randomization. Table 3-3 Various SPLs as they affect humans (Hamby, 2004 and EPA 2012) to show a frame of reference for the experimental treatments.

Table 3-2 Letters assigned to treatments to be used for Latin Square Randomizations

Treatments			
Frequency (kHz)	**Intensity (dB)**		
	80	**100**	**120**
1	A	B	C
8	D	E	F

Table 3-3 Reference SPLs

	SPL (dB)
Whisper	40
Normal conversation at 1 m	60
EPA-identified maximum to protect against hearing loss and other disruptive effects from noise, such as sleep disturbance, stress, learning detriment, etc.	70
Beginning of hearing damage, earplugs should be worn	85
Hearing damage (possible)	120

3.2.2 Experimental Design

The experimental design was a Randomized 6x6 Latin Square presented in Table 3-4. This ensured that each animal received each treatment once over the six weeks of testing. The number of experimental units was increased by using three replications of the Latin squares.

Table 3-4 Randomized 6 x 6 Latin Square indicating which treatment animals would receive a given week.

Animal Number	**Week**					
	1	**2**	**3**	**4**	**5**	**6**
1	D	C	E	B	A	F
2	F	E	A	D	C	B
3	C	B	D	A	F	E
4	B	A	C	F	E	D
5	E	D	F	C	B	A
6	A	F	B	E	D	C
7	A	C	F	D	E	B
8	F	B	E	C	D	A
9	E	A	D	B	C	F
10	C	E	B	F	A	D
11	D	F	C	A	B	E
12	B	D	A	E	F	C
13	E	B	D	C	A	F
14	F	C	E	D	B	A
15	D	A	C	B	F	E
16	B	E	A	F	D	C
17	A	D	F	E	C	B
18	C	F	B	A	E	D

Data was analyzed using the PROCMIXED function in SAS® (9.3, SAS Institute Inc., 2002-2008 Cary, NC, U.S.A). Use of this function provided the ability to analyze all three testing procedures together. The data was tested for significance based on the different frequencies, intensities, periods, weeks, and also tested for all possible interactions.

The averages of the values for heart rate and eye temperature for each period were used in the analysis. The startle response, i.e. animal movement, was measured using changes in the scale readings for the animal's weight; therefore, the standard deviations of the weights were compared for the three periods. By tapping into the scale head and sampling the load cell outputs at a high frequency rate, the variability in the measured weights can be considered an indicator of movement. A larger standard deviation value is considered an indicator of more movement.

The differences in each stress measurement between periods were also examined in order to provide a more detailed analysis. These differences are important because a significant difference between treatments for period differences would indicate stress occurred. The differences between period one-period two and period three-period two were calculated using the averages already recorded. This data was also analyzed using the PROCMIXED function in SAS® (9.3, SAS Institute Inc., 2002-2008 Cary, NC, U.S.A). Data was tested for the effects of intensity, frequency, week, and the interaction between intensity and frequency.

3.2.3 Heart Rate Testing

Heart Rate was recorded using a Polar Equine RS800CX Science system (Polar, 2011). Each animal was equipped with the elastic strap that attaches the transmitters to the animals (Figure 3-8 and Figure 3-9) and then were given an hour to become accustomed to the equipment, as recommended by Borell et al (2007).

Figure 3-8 A feeder that has been equipped with the elastic strap

Heart rate data for each animal over the period of the experiment was stored on the Polar Equine RS800CX Science (Polar, 2011) and then imported into Excel (Microsoft, 2010). The heart rate values for each treatment were spilt into the three data analysis periods: pre-treatment (P1), during-treatment (P2), and post-treatment (P3). The average heart rate for each period was calculated and used in the statistical analysis.

Figure 3-9 Snapping mechanism for attaching the Wearlink® transmitter to the strap.

3.2.3.1 Heart Rate Monitors

The Polar Equine RS800CX Science (Polar, 2011) (Figure 3-10) is a system used to remotely measure the heart rate of horses, but can be adapted to work with cattle as well. Each system is composed of a training computer that records the data, a Wearlink® transmitter with strap, and the computer software to examine the electrocardiogram. Each animal must be equipped with both the training computer and transmitter to measure heart rate. The training computers cannot be shared between multiple transmitters because they are programmed to communicate with each other on an individual frequency. This allows multiple units to be in range of each other without cross communication.

Figure 3-10 Training computer for the Polar Equine RS800CX heart rate monitoring system

3.2.4 Movement

Sudden movement by the animals, referred to as a startle response was measured as an indicator of behavioral change in response to the sound stimuli. The chute used during the experiment was suspended on two load cells. The changes in voltage output from the load cells are typically used to measure the weight of the animals. Movement data was recorded by connecting an output port on the scale head to a computer via the serial port. Use of the COM Port Toolkit 3.8 (Michael Golikov, 2007) configured the data acquisition system to record the weight measurement five times per second and export the measurements into Excel (Microsoft, 2010) for later analysis. Similar to the other two methods of stress analysis, the weight measurements were divided into the three periods. The standard deviation of the weight measurement during each period was calculated and then used for comparison.

3.2.4.1 Infrared Thermal Camera

The camera used during the experiment was the Flir systems ThermaCAM SC640 (Figure 3-11) with a 22mm lens (Flir, 2006). The camera was programmed to record one frame per 1.3 seconds. The camera records all of the images on a secured digital (SD) card for later analysis.

Figure 3-11 ThermaCAM SC640 used to take the infrared photos of the steer’s eyes.

3.2.5 Eye Temperature

The eye temperature was measured with an infrared camera as a secondary indicator of physiological response to sound stimuli. The maximum eye temperature was measured within the area of the medial posterior palpebral border of the lower eyelid and the lacrimal caruncle (Figure 3-12 and Figure 3-13) (Stewart, 2007b). The infrared camera was positioned to take pictures of a steer’s eye while it was restrained for the experiment. The camera was placed approximately one meter (3.3ft) perpendicular to the chute as shown in Figure 3-7. Infrared pictures of the eye were recorded every 1.3 seconds during the three minute experiment. ThermaCAM Researcher Pro 2.10 (Flir Systems, Inc. 1999-2013) was used to find the eye temperature in the recommended region for each image recorded. The temperature values were stored in Excel (Microsoft, 2010) and then divided into the three periods for analysis. The average eye temperature during each period was calculated and then used for the statistical analysis.

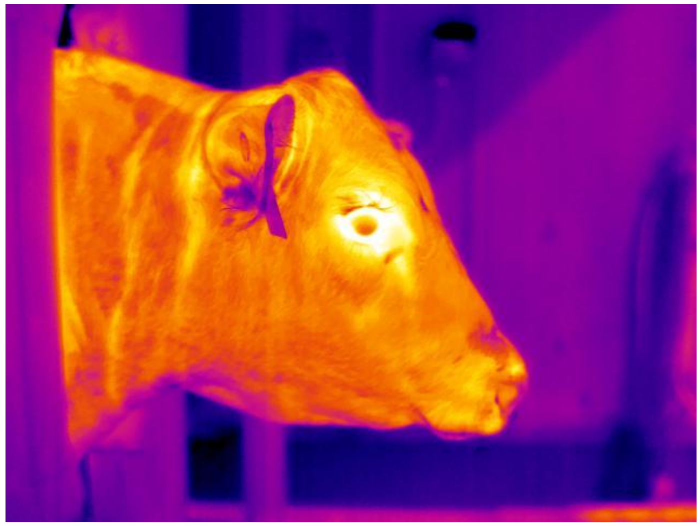

Figure 3-12 An infrared image of a beef eye region.

Figure 3-13 The ThermaCAM recording the eye temperature during an experiment.

CHAPTER 4 Results and Discussion

4.1 Sound Testing Results

Initial sound testing of the typical squeeze chute resulted in SPL's which ranged from 99-115 dB at two frequencies of one and eight kilohertz. The loudest sounds were measured coming from the head gate area which was the closest to the animal's ears while they were in the chute. The operator was standing in the area of the head gate during operation of the equipment, making them also subject to the highest level during operation. These measurements were the basis of the treatment levels used during stress testing.

Table 4-1 Results of sound testing of the CattleMaster.

Noise Source	Intensity (dB)	Frequency (kHz)
Head Gate	114.6	8
Tailgate	109.8	1
Whole Chute	99	8
Side Access Door	101.4	8
Squeeze Action	99.9	1

4.2 Stress Testing Results

4.2.1 Period Length

Initially the data was analyzed using the full one minute period lengths. However, no significant difference was observed for any treatments. It was concluded that shorter period lengths should be examined in order to determine whether significant stress responses of a more transient nature might have occurred. Movement was analyzed for period lengths of 30, 20, 10, 5, and 2.5 seconds. Period length was shortened to 20 seconds before a significant difference was observed in the movement data. Heart rate was analyzed for period lengths of 30, 20, and 10 seconds. A significant difference was not observed as a result of shortening the period difference for heart rate. The period length of 20 seconds was used to report the final data analysis. Period one was the 20 seconds directly prior to treatment, period two was 20 seconds following treatment, and period three was 20 seconds starting one minute after treatment was applied.

4.2.2 Heart Rate

Figure 4-1 shows the raw data downloaded from the heart rate monitors for steer number 104. Using Excel the average of the heart rate during each period was calculated. Table 4-2 shows the calculated average heart rates for one of the steers for a period length of 20 seconds. These calculations were completed for all 18 steers.

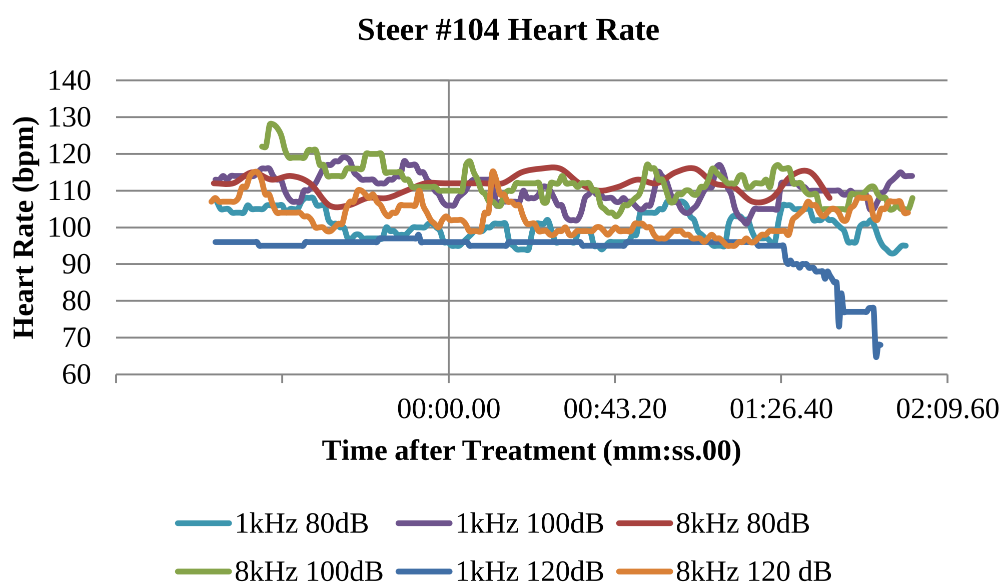

Figure 4-1 Heart Rate Raw Data for Steer 104

Table 4-2 Calculated average heart rate for Animal 104

Animal	Week	TRT	Int	Freq	Per	Average HR
104	1	D	80	8	1	110.8
104	1	D	80	8	2	114.2
104	1	D	80	8	3	110.0
104	2	C	120	1	1	96.6
104	2	C	120	1	2	95.5
104	2	C	120	1	3	96.0
104	3	E	100	8	1	112.2
104	3	E	100	8	2	110.7
104	3	E	100	8	3	112.0
104	4	B	100	1	1	113.7
104	4	B	100	1	2	109.3
104	4	B	100	1	3	107.4
104	5	A	80	1	1	98.7
104	5	A	80	1	2	97.3
104	5	A	80	1	3	99.4
104	6	F	120	8	1	105.2
104	6	F	120	8	2	104.3
104	6	F	120	8	3	96.7

None of the treatments tested resulted in a significant difference in average heart rate at a 95 % confidence level. The results of the type 3 test of fixed effects are presented in Table 4-3. The effect of week on the average heart rate was significant. The polynomial contrasts function in SAS® was used to determine that there was a significant linear effect for weeks. The results suggest that there is a negative slope of means (Figure 4-2), which indicated a decreasing heart rate over the six weeks. This shows that the animals became acclimated to the noise and being in the chute over the time of the experiment.

Table 4-3 Type 3 fixed effects for average heart rate.

	Pr > F
Int	0.8022
Freq	0.7624
Int*Freq	0.3467
Per	0.6832
Int*Freq*Per	0.0877
Week	0.0269

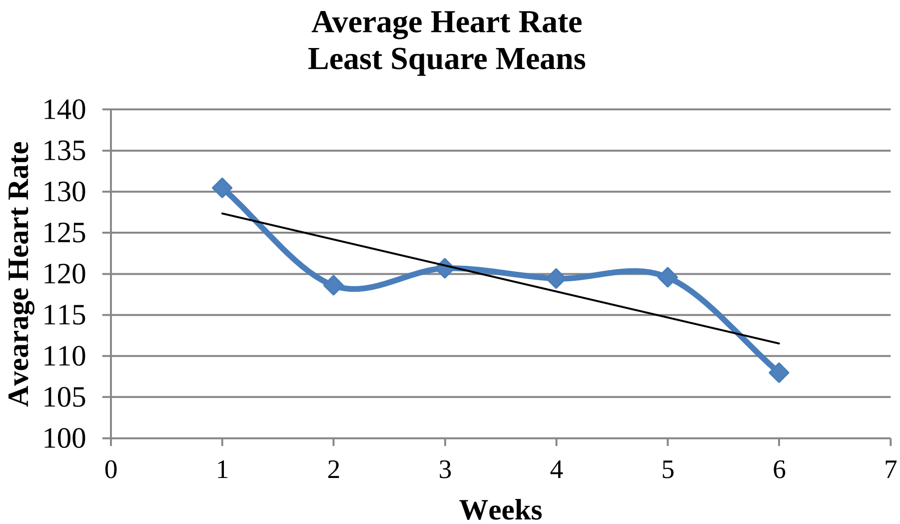

Figure 4-2 Least square means of average heart rate

Table 4-4 shows the calculated period differences for one of the animals. Similar to the average heart rate, these values were calculated for all 18 animals and then input into SAS®.

Table 4-4 Period differences for steer 104

Animal	Week	TRT	Int	Freq	P2-P1	P3-P2
104	1	D	80	8	3.4	-4.2
104	2	C	120	1	-1.05882	0.5
104	3	E	100	8	-1.52101	1.333333
104	4	B	100	1	-4.37282	-1.85714
104	5	A	80	1	-1.33333	2.066667
104	6	F	120	8	-0.85714	-7.66667

The results of the Type 3 Test of Fixed effects are presented in Tables 4-5 and 4-6. The results indicate there was no significant difference in the heart rate period difference values for the intensity*frequency interaction, intensity, or frequency at a 95% confidence level. This suggests that none of the treatments had a significant effect than the other on the stress of level of cattle based on heart rate.

Table 4-5 Type 3 Test of Fixed Effects results P2-P1

	Pr>F
Intensity	0.5082
Frequency	0.4873
Intensity*Frequency	0.2531
Week	0.6692

Table 4-6 Type 3 Test of Fixed Effects results P3-P2

	Pr > F
Intensity	0.1266
Frequency	0.2865
Intensity*Frequency	0.3123
Week	0.4129

Waynert et al. (1998) indicated the cattle's average heart rate during the one minute prior to treatment was 92 bpm and 99 bpm during the one minute treatment period. The average heart rate in this experiment measured one minute prior to treatment was approximately 120 bpm. The normal resting heart rate of cattle is 48-84 bpm (Detweiler et al., 2004). The average heart rates of the animals in this experiment were already elevated above normal resting rate prior to the start of the experiment. The animals' above normal heart rates may have limited any potential change in heart rate as a result of noise stimulus. Two possible causes of the elevated heart rate could have been the animal handling procedure prior to the noise treatment or heat stress. The cattle were worked in the early morning in an effort to minimize heat stress, however temperatures outside were still above the cattle's thermal neutral zone. Table 4-4 lists the daily highs and lows for the days on which testing occurred. Weather data was retrieved from a National Oceanic and Atmospheric Administration (NOAA) weather station. The NOAA weather station is located at the Beef Unit in Versailles, KY.

Table 4-7 Air Temperature on testing days

Test Date	High Temp (°F)	Low Temp (°F)
7/24/2012	90.3	76.1
8/7/2012	86.7	65.8
8/14/2012	80.6	66.7
8/21/2012	80.8	52
9/4/2012	84.6	70
9/11/2012	77.7	49.5

4.2.3 Movement

Figure 4-3 shows an example of the raw weight data collected for one of the steers. Animal movement was measured as the variation in weights recorded during the experiment. Specifically, we used the standard deviation of weights recorded at 200 millisecond intervals for the duration of the three minute experiment. Similar to the heart rate analysis, movement was also analyzed using a time period of 20 seconds. A larger standard deviation indicated more movement occurred. Table 4-8 shows an example of the calculated standard deviations for animal 104. These same calculations were repeated for all 18 animals before analysis in SAS®.

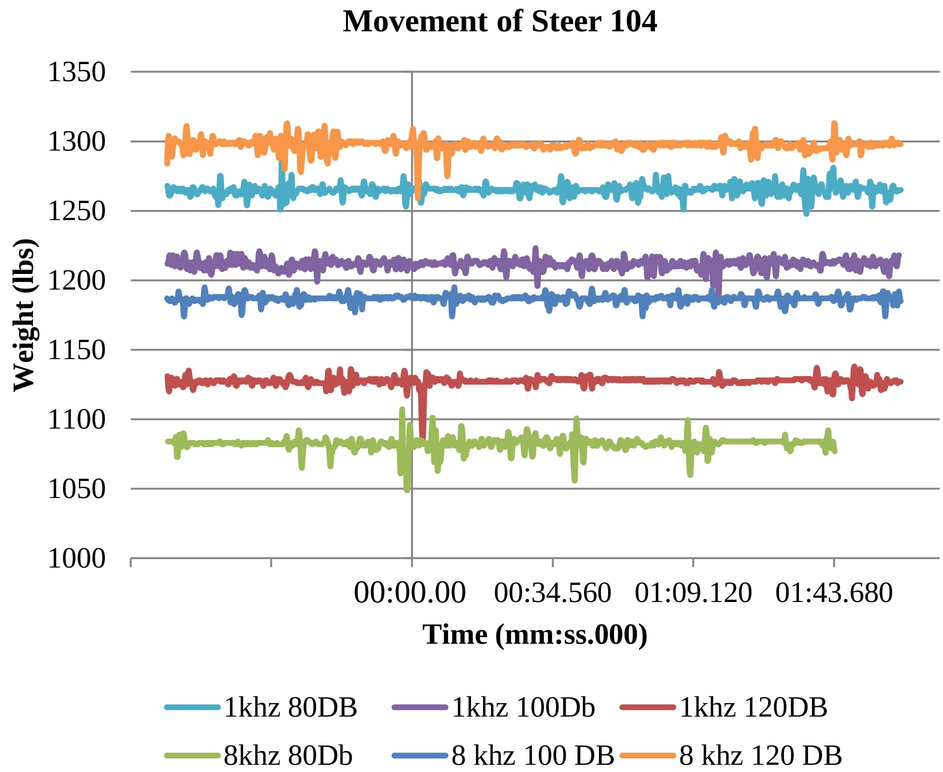

Figure 4-3 Movement raw data for steer 104 with noise stimulus at time = 0

Table 4-8 Standard deviation calculations for animal 104

Animal	Week	TRT	Int	Freq	Per	Movement (Std Dev.)
104	1	D	80	8	1	7.640968
104	1	D	80	8	2	5.2650944
104	1	D	80	8	3	4.7587906
104	2	C	120	1	1	3.1481414
104	2	C	120	1	2	6.4804187
104	2	C	120	1	3	1.2505871
104	3	E	100	8	1	2.2459221
104	3	E	100	8	2	2.3350037
104	3	E	100	8	3	1.9223925
104	4	B	100	1	1	2.3536157
104	4	B	100	1	2	2.1265761
104	4	B	100	1	3	4.5857731
104	5	A	80	1	1	2.9920785
104	5	A	80	1	2	2.0921315
104	5	A	80	1	3	3.2558655
104	6	F	120	8	1	2.7947485
104	6	F	120	8	2	6.2520119
104	6	F	120	8	3	1.6929112

There is a significant difference in the movement of the animal as a result of intensity (Table 4-9). Use of the polynomial contrasts function in SAS® indicated that there is a significant linear effect of intensity (Figure 4-4). As intensity increased the standard deviation least square means increased. This indicated that the higher intensities produced more movement which may also increase the animals' physiological stress level.

Table 4-9 Type 3 test of fixed effects of movement

	Pr > F
Int	0.0125
Freq	0.6707
Int*Freq	0.5541
Per	0.6489
Int*Freq*Per	0.3377
Week	0.3748

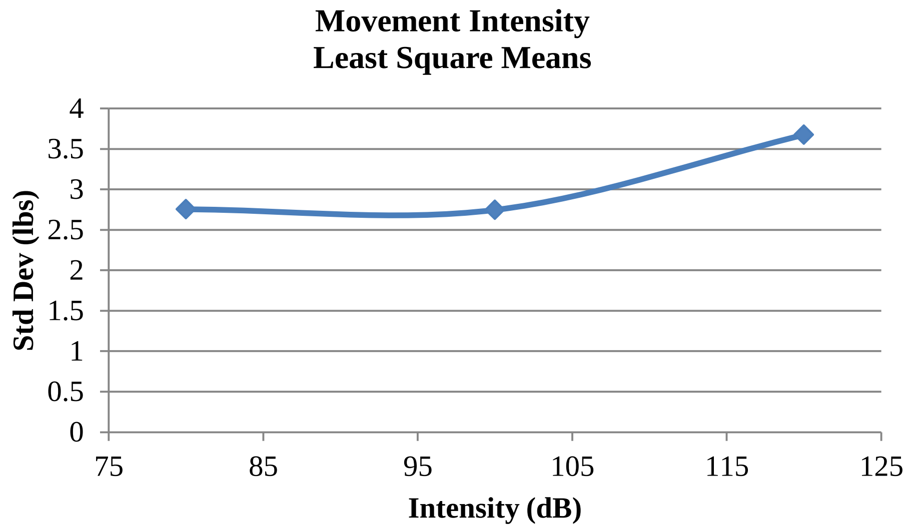

Figure 4-4 Graph of the movement intensity least square means

The period differences for movement were significantly different based for both frequency and intensity (Table 4-10). Using the polynomial contrasts function in SAS® it was found that there is a significant linear effect of intensity and frequency. As the intensity increased there is more movement in period two compared to periods one and three (Figure 4-5). Eight kilohertz frequency resulted in a smaller period difference than one kilohertz (Figure 4-6). As with intensity there is more movement during period two than periods one or three. This increase in movement during period two indicates a stress response occurred as a result of the treatments.

Table 4-10 Type 3 test of fixed effects for movement period differences

	Pr>F	
	P2-P1	**P3-P2**
Int	0.0639	0.0064
Freq	0.0646	0.0596
Freq*Int	0.3622	0.1411
Week	0.4313	0.3864

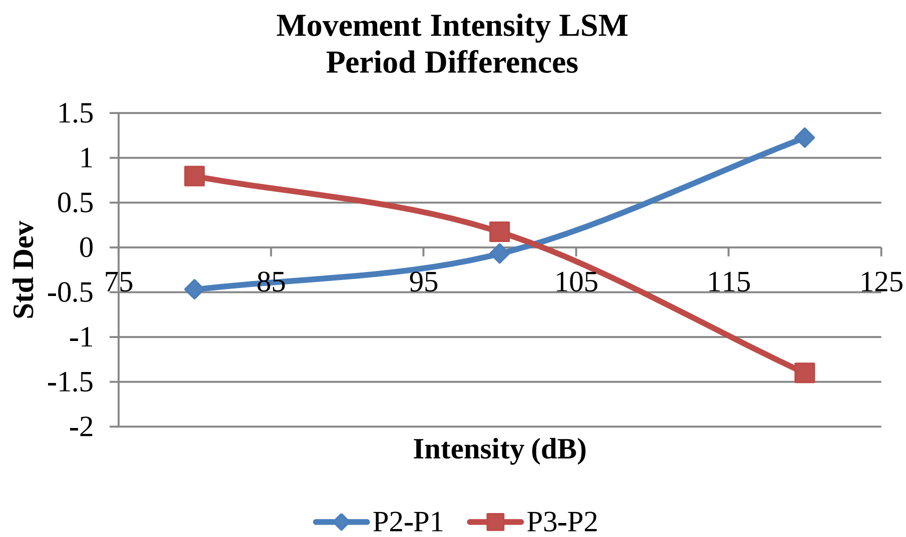

Figure 4-5 LSM comparison for movement intensity period difference

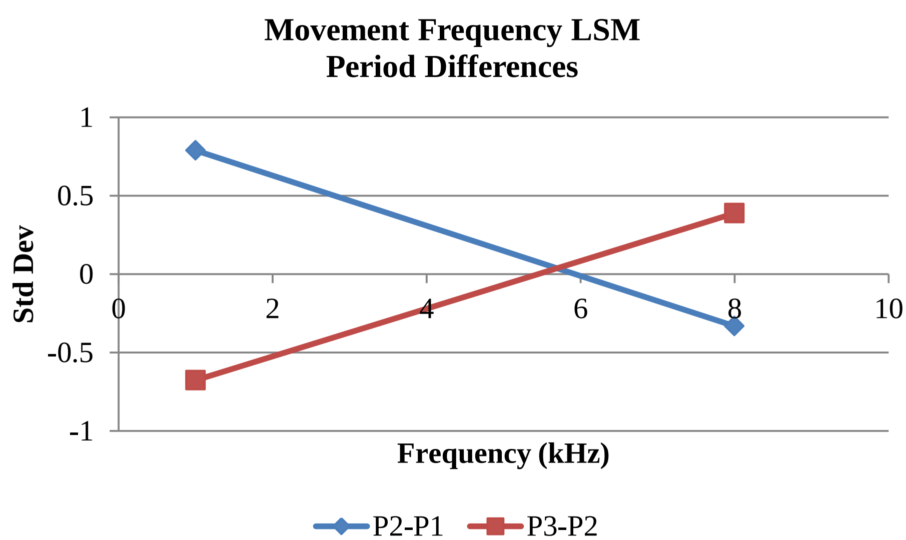

Figure 4-6 LSM comparison for movement frequency period difference

Waynert et al. (1998) also used movement as a measure of stress during his test of how noise stimuli affect cattle. The noise stimuli for this experiment averaged 85 dB and were compared against animals held in silence. The noise stimuli caused the cattle to have a significantly higher amount of movement as compared to the cattle handled in silence.

4.2.4 Eye Temperature

The eye temperature analysis for this experiment does not indicate a measurable stress response as a result of noise stimuli. One possible reason for the lack of measurable response could have been a limitation of available equipment. It is unknown if a measurable difference could have been recorded if a zoom lens with greater magnifying capability was used. Another limitation to measuring the eye temperature was the head movement of the cattle. The camera was set up on a tri-pod and was moved up and down in response to head movement in order to capture the images without disturbing the animal. This proved to be a difficult task and thus, it was not always possible to view the eye in a location where a useable image could be captured by the camera. It appears that in order to accurately measure eye temperature, the head needed to be perpendicular to the camera lens. The animals' head movement from side to side made it hard to record an accurate eye temperature in some images.

Eye temperature is a relatively new method of stress measurement. As such, there are some uncertainties associated with its use as a stress indicator for a noise stimulus. No other experiments were found in the literature which used eye temperature as a measure of stress in relation to noise stimuli. This was in part, one of the reasons this methodology was chosen as part of this work. It should be noted that when Stewart et al (2007b) used eye temperature as a measurement of stress, he was actually invoking pain on the animals and measuring the response. This suggests that perhaps the stress risor must be more acute than a highly transient sound event, and that more testing should be done to validate eye temperature as a stress indicator in a response to a "fear" stress rather than pain.

CHAPTER 5 Conclusions

The squeeze chute is typically not a piece of equipment which receives daily use at a cattle operation. However, reducing stress during handling will provide advantages of increasing productivity and maintaining meat quality (Grandin, 1998). Sound pressure level (SPL) is known to cause stress in cattle but is often overlooked as a potential source of fear for cattle when designing handling equipment.

Measuring heart rate did not produce a significant difference based on the different treatment levels. Waynert et al. (1998) has proven that heart rate can produce a measurable response as a result to noise stimuli. The already elevated heart rate of the steers prior to the start of the experiment may have masked or limited any measurable increase in heart rate as a result of the treatment, thereby obscuring any possible comparisons at the stated treatment levels. The reason for the elevated heart rates is unknown.

A measurable response as a result of a short term noise stressor was observed from the movement data. There was a significant difference of the movement standard deviation based on the intensity of the noise stimuli. Increasing intensity correlated with increased movement, therefore a lower intensity produces less stress response from the animals based on movement.

Eye temperature did not appear to have a measurable response to stress as a result of noise stimuli. Additional testing needs to be completed to determine if eye temperature is an indicator of stress as a result to noise stimuli, and also to determine minimum technical requirements for the data gathering system.

Movement showed that lowering the intensity of the noise lowered the stress of the steers. However, before a recommended sound pressure level design limit can be made, it is felt that this finding should be further correlated with a measurable physiological response to the noise treatments. Waynert et al. (1998) also showed movement was a measure of stress response in beef cattle as a result noise stimuli.

CHAPTER 6 Future Work

Future work relating to this experiment should continue toward development of a standardized SPL to be used during the design of handling equipment. A broader range of frequencies should be tested to gain a better understanding of the stress response to SPL. Further experiments should include a broader range of frequencies to better illustrate the understanding of its effects on the stress levels.

Additional testing involving the measurement of heart rate as affected by the different treatments should be undertaken. It has been shown that heart rate is an indicator of stress from a noise stimulus. The results of this project indicate that more care should be taken to reduce stress and not raise the heart rate prior to the treatment being applied.

Eye temperature is still a fairly new measurement method for stress in cattle. More testing should be done using this method to verify that it is an indicator of stress. It is recommended to use a lens which would record images closer to the eye in greater thermal detail than could be obtained in this study. This increased resolution would allow the camera to be focused more tightly on the area of concern which could potentially allow the camera to pick up smaller changes in eye temperature. However, zooming the camera in to take a tighter image of the eye will also complicate the issues associated with the animal's head movement. Some procedures for limiting head movement or for enabling the camera to follow head movement will need to be developed. Further testing may also provide insight into the different types of stress i.e., pain induced as compared to transient noise events.

Most importantly, future work should design and test different ideas for sound reduction on the actual handling equipment as a means of verifying the results of the statistical models. The goal would be to use the results of the various analyses to reduce the sound pressure level (and to reduce stress) without adding significant cost to the equipment.

Appendix A. Tarter CattleMaster features

According to Tarter Farm and Ranch Equipment (2011) the key features of the Cattlemaster include:

- Heavy-duty, all-steel welded construction
- Modular design allows easy addition of optional accessories such as palpation cage, tailgates, and alleyways
- Available with manual or automatic head gate
- Easily adjusts to various cattle sizes/breeds
- User-friendly ergonomic design
- Single-lever squeeze mechanism
- Drop-down horseshoes for easy top-side access, as well as the fold-down and fully removable bottom panels
- Side exit door for larger animal exit
- Sliding vertical tailgate with stop
- Bottom adjustments with multiple settings for various size animals
- Durable powder coat finish in red only

Appendix B. SAS® Code

B.1 Mixed procedure syntax for 6x6 latin square

```
proc mixed;
class animal int freq week per;
model HR = int|freq|per week week*per/ddfm=kr;
random animal;
repeated per/sub=animal(week) type=ar(1);
lsmeans int|freq|per week week*per;
contrast 'Linear HR' int -1 0 1;
contrast 'Quad HR' int 1 -2 1;
run;
quit;
```

In the Model statement HR was interchanged with movement and eye temperature to run the other analysis.

B.2 Mixed procedure for period differences

```
proc mixed;
class animal freq int  week;
model P2_P1 = int|freq week /ddfm=kr;
repeated week/sub=animal type=cs;
lsmeans int|freq week;
contrast 'Linear Int' int -1 0 1  ;
contrast 'Quad Int' int 1 -2 1 ;
contrast 'Linear Freq' freq -1 1;
proc mixed;
class animal freq int  week;
model P3_P2 = int|freq week /ddfm=kr;
repeated week/sub=animal type=cs;
lsmeans int|freq week;
contrast 'Linear Int' int -1 0 1 ;
contrast 'Quad Int' int 1 -2 1 ;
contrast 'Linear Freq' freq -1 1;
run;
quit;
```

Appendix C. Statistical Results for 1 minute period length

C.1 Heart Rate

Table 6-1 Model information - SAS® output for Heart Rate using a Randomized 6x6 latin square design

Model Information	
Data Set	WORK.DATA1
Dependent Variable	HR
Covariance Structures	Variance Components, Autoregressive
Subject Effect	Animal(Week)
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Kenward-Roger
Degrees of Freedom Method	Kenward-Roger

Class Level Information

Class	Levels	Values
Animal	18	104 125 154 156 161 167 169 178 180 196 240 251 272 279 339 370 392 394
Int	3	80 100 120
Freq	2	1 8
Week	6	1 2 3 4 5 6
Per	3	1 2 3

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
Animal		64.8473
AR(1)	**Animal(Week)**	0.8178
Residual		362.35

Fit Statistics	
-2 Res Log Likelihood	2212.7
AIC (smaller is better)	2218.7
AICC (smaller is better)	2218.8
BIC (smaller is better)	2221.4

Table 6-2 SAS® Type III of fixed effects for Heart Rate

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Int	2	79	0.05	0.9521
Freq	1	79.8	0.25	0.6208
Int*Freq	2	79.1	2.58	0.0823
Per	2	167	2.14	0.1208
Int*Per	4	167	0.82	0.5156
Freq*Per	2	166	0.68	0.5065
Int*Freq*Per	4	167	1.33	0.2619
Week	5	80.3	3.49	0.0066
Week*Per	10	167	0.34	0.9705

Table 6-3 SAS® Contrast Result for heart rate

Contrasts				
Label	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Linear HR	1	82.7	11.77	0.0009
Quad HR	1	83.2	0.17	0.6795
Cubic HR	1	79.6	4.75	0.0323
Quartic HR	1	78.1	0.10	0.7547

Table 6-4 SAS® Least Square Means Results for Heart Rate

Least Squares Means									
Effect	**Int**	**Freq**	**Week**	**Per**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Int	**80**				118.70	3.4951	51.5	33.96	<.0001
Int	**100**				119.03	3.5106	52.4	33.90	<.0001
Int	**120**				119.96	3.4953	51.5	34.32	<.0001
Freq		**1**			120.07	3.0764	34.7	39.03	<.0001
Freq		**8**			118.38	3.0533	33.8	38.77	<.0001
Int*Freq	**80**	**1**			124.36	4.5750	85.6	27.18	<.0001
Int*Freq	**80**	**8**			113.05	4.5538	84.1	24.82	<.0001
Int*Freq	**100**	**1**			115.25	4.6046	87.6	25.03	<.0001
Int*Freq	**100**	**8**			122.80	4.5620	84.7	26.92	<.0001
Int*Freq	**120**	**1**			120.60	4.5771	85.7	26.35	<.0001
Int*Freq	**120**	**8**			119.31	4.5539	84.1	26.20	<.0001
Per				**1**	121.23	2.7598	23.3	43.93	<.0001
Per				**2**	118.61	2.6378	19.6	44.97	<.0001
Per				**3**	117.84	2.6400	19.7	44.64	<.0001
Int*Per	**80**			**1**	120.92	3.8540	74.2	31.37	<.0001
Int*Per	**80**			**2**	117.77	3.6970	63.9	31.85	<.0001
Int*Per	**80**			**3**	117.43	3.6970	63.9	31.76	<.0001
Int*Per	**100**			**1**	122.14	3.9662	81.9	30.80	<.0001
Int*Per	**100**			**2**	117.32	3.6970	63.9	31.73	<.0001
Int*Per	**100**			**3**	117.62	3.7114	64.8	31.69	<.0001
Int*Per	**120**			**1**	120.63	3.8557	74.3	31.29	<.0001
Int*Per	**120**			**2**	120.76	3.6970	63.9	32.66	<.0001
Int*Per	**120**			**3**	118.48	3.6970	63.9	32.05	<.0001
Freq*Per		**1**		**1**	121.47	3.4838	55.3	34.87	<.0001
Freq*Per		**1**		**2**	120.16	3.2114	41.1	37.42	<.0001
Freq*Per		**1**		**3**	118.59	3.2187	41.4	36.84	<.0001

Least Squares Means									
Effect	**Int**	**Freq**	**Week**	**Per**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Freq*Per		**8**		**1**	120.99	3.3022	45.6	36.64	<.0001
Freq*Per		**8**		**2**	117.07	3.2114	41.1	36.45	<.0001
Freq*Per		**8**		**3**	117.09	3.2114	41.1	36.46	<.0001
Int*Freq*Per	**80**	**1**		**1**	123.27	5.1986	133	23.71	<.0001
Int*Freq*Per	**80**	**1**		**2**	124.30	4.8717	108	25.51	<.0001
Int*Freq*Per	**80**	**1**		**3**	125.52	4.8717	108	25.76	<.0001
Int*Freq*Per	**80**	**8**		**1**	118.56	5.0285	120	23.58	<.0001
Int*Freq*Per	**80**	**8**		**2**	111.23	4.8717	108	22.83	<.0001
Int*Freq*Per	**80**	**8**		**3**	109.34	4.8717	108	22.44	<.0001
Int*Freq*Per	**100**	**1**		**1**	118.97	5.3902	148	22.07	<.0001
Int*Freq*Per	**100**	**1**		**2**	114.42	4.8717	108	23.49	<.0001
Int*Freq*Per	**100**	**1**		**3**	112.37	4.9151	111	22.86	<.0001
Int*Freq*Per	**100**	**8**		**1**	125.32	5.0945	125	24.60	<.0001
Int*Freq*Per	**100**	**8**		**2**	120.23	4.8717	108	24.68	<.0001
Int*Freq*Per	**100**	**8**		**3**	122.86	4.8717	108	25.22	<.0001
Int*Freq*Per	**120**	**1**		**1**	122.16	5.2149	135	23.43	<.0001
Int*Freq*Per	**120**	**1**		**2**	121.77	4.8717	108	24.99	<.0001
Int*Freq*Per	**120**	**1**		**3**	117.88	4.8717	108	24.20	<.0001
Int*Freq*Per	**120**	**8**		**1**	119.10	5.0285	120	23.69	<.0001
Int*Freq*Per	**120**	**8**		**2**	119.74	4.8717	108	24.58	<.0001
Int*Freq*Per	**120**	**8**		**3**	119.07	4.8717	108	24.44	<.0001
Week			**1**		130.78	4.6921	93.8	27.87	<.0001
Week			**2**		117.82	4.5401	83.2	25.95	<.0001
Week			**3**		122.12	4.5928	86.8	26.59	<.0001
Week			**4**		118.84	4.5401	83.2	26.18	<.0001
Week			**5**		119.55	4.5349	82.8	26.36	<.0001
Week			**6**		106.26	4.6034	87.6	23.08	<.0001

Least Squares Means									
Effect	**Int**	**Freq**	**Week**	**Per**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Week*Per			**1**	**1**	129.40	6.0654	196	21.33	<.0001
Week*Per			**1**	**2**	131.67	4.8717	108	27.03	<.0001
Week*Per			**1**	**3**	131.27	4.8717	108	26.95	<.0001
Week*Per			**2**	**1**	120.91	4.8717	108	24.82	<.0001
Week*Per			**2**	**2**	116.57	4.8717	108	23.93	<.0001
Week*Per			**2**	**3**	115.97	4.9151	111	23.59	<.0001
Week*Per			**3**	**1**	124.79	5.3380	144	23.38	<.0001
Week*Per			**3**	**2**	120.57	4.8717	108	24.75	<.0001
Week*Per			**3**	**3**	121.01	4.8717	108	24.84	<.0001
Week*Per			**4**	**1**	121.29	4.9157	112	24.67	<.0001
Week*Per			**4**	**2**	117.14	4.8717	108	24.05	<.0001
Week*Per			**4**	**3**	118.09	4.8717	108	24.24	<.0001
Week*Per			**5**	**1**	122.76	4.8717	108	25.20	<.0001
Week*Per			**5**	**2**	119.27	4.8717	108	24.48	<.0001
Week*Per			**5**	**3**	116.63	4.8717	108	23.94	<.0001
Week*Per			**6**	**1**	108.23	5.4194	151	19.97	<.0001
Week*Per			**6**	**2**	106.47	4.8717	108	21.86	<.0001
Week*Per			**6**	**3**	104.08	4.8717	108	21.36	<.0001

C.2 Eye Temperature

Table 6-5 Model information - SAS® output for Eye Temperature using a Randomized 6x6 latin square design

Model Information		
Data Set		WORK.DATA2
Dependent Variable		eyetemp
Covariance Structures		Variance Components, Autoregressive
Subject Effect		Animal(Week)
Estimation Method		REML
Residual Variance Method		Profile
Fixed Effects SE Method		Kenward-Roger
Degrees of Freedom Method		Kenward-Roger
Class Level Information		
Class	**Levels**	**Values**
Animal	18	104 125 154 156 161 167 169 178 180 196 240 251 272 279 339 370 392 394
Int	3	80 100 120
Freq	2	1 8
Week	6	1 2 3 4 5 6
Per	3	1 2 3
Covariance Parameter Estimates		
Cov Parm	**Subject**	**Estimate**
Animal		0.3595
AR(1)	**Animal(Week)**	0.6146
Residual		14.9798

Fit Statistics	
-2 Res Log Likelihood	1500.1
AIC (smaller is better)	1506.1
AICC (smaller is better)	1506.2
BIC (smaller is better)	1508.8

Table 6-6 SAS® Type III of fixed effects for eye temperature

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Int	2	90.8	0.72	0.4882
Freq	1	91.4	0.23	0.6313
Int*Freq	2	90.5	1.08	0.3450
Per	2	187	0.29	0.7513
Int*Per	4	188	1.17	0.3255
Freq*Per	2	187	1.61	0.2032
Int*Freq*Per	4	188	0.92	0.4554
Week	5	90.9	4.21	0.0017
Week*Per	10	189	1.00	0.4470

Table 6-7 SAS® Contrast result for eye temperature

Contrasts				
Label	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Linear HR	1	91.6	11.76	0.0009
Quad HR	1	91.3	1.49	0.2250
Cubic HR	1	90	0.73	0.3948
Quartic HR	1	90.6	6.76	0.0109

Table 6-8 SAS® least square means results for eye temperature

Least Squares Means									
Effect	**Int**	**Freq**	**Week**	**Per**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Int	**80**				95.4893	0.5659	83.3	168.73	<.0001
Int	**100**				95.5073	0.5546	80.6	172.20	<.0001
Int	**120**				94.6848	0.5758	84.5	164.43	<.0001
Freq		**1**			95.0749	0.4629	51.6	205.38	<.0001
Freq		**8**			95.3793	0.4750	53.3	200.80	<.0001
Int*Freq	**80**	**1**			95.6711	0.7742	109	123.57	<.0001
Int*Freq	**80**	**8**			95.3074	0.8011	111	118.97	<.0001
Int*Freq	**100**	**1**			95.6830	0.7718	108	123.97	<.0001
Int*Freq	**100**	**8**			95.3315	0.7712	108	123.62	<.0001
Int*Freq	**120**	**1**			93.8706	0.7835	113	119.81	<.0001
Int*Freq	**120**	**8**			95.4990	0.8196	107	116.52	<.0001
Per				**1**	95.4035	0.4103	33.6	232.52	<.0001
Per				**2**	95.1597	0.4070	32.7	233.83	<.0001
Per				**3**	95.1181	0.4105	33.6	231.73	<.0001
Int*Per	**80**			**1**	95.3996	0.6765	147	141.03	<.0001
Int*Per	**80**			**2**	95.5160	0.6760	147	141.30	<.0001
Int*Per	**80**			**3**	95.5522	0.6922	155	138.05	<.0001
Int*Per	**100**			**1**	95.2342	0.6666	145	142.88	<.0001
Int*Per	**100**			**2**	95.6176	0.6648	145	143.82	<.0001
Int*Per	**100**			**3**	95.6700	0.6604	142	144.88	<.0001
Int*Per	**120**			**1**	95.5768	0.7018	155	136.19	<.0001
Int*Per	**120**			**2**	94.3456	0.6863	149	137.48	<.0001
Int*Per	**120**			**3**	94.1321	0.6939	151	135.66	<.0001
Freq*Per		**1**		**1**	95.6594	0.5629	101	169.94	<.0001
Freq*Per		**1**		**2**	94.8175	0.5477	93.6	173.11	<.0001
Freq*Per		**1**		**3**	94.7478	0.5555	97.4	170.56	<.0001

Least Squares Means									
Effect	**Int**	**Freq**	**Week**	**Per**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Freq*Per		**8**		**1**	95.1476	0.5623	96.5	169.20	<.0001
Freq*Per		**8**		**2**	95.5020	0.5675	99.5	168.27	<.0001
Freq*Per		**8**		**3**	95.4884	0.5701	101	167.49	<.0001
Int*Freq*Per	**80**	**1**		**1**	95.6259	0.9409	189	101.64	<.0001
Int*Freq*Per	**80**	**1**		**2**	95.7183	0.9231	182	103.69	<.0001
Int*Freq*Per	**80**	**1**		**3**	95.6692	0.9408	189	101.69	<.0001
Int*Freq*Per	**80**	**8**		**1**	95.1733	0.9519	182	99.98	<.0001
Int*Freq*Per	**80**	**8**		**2**	95.3136	0.9673	191	98.53	<.0001
Int*Freq*Per	**80**	**8**		**3**	95.4352	0.9945	198	95.96	<.0001
Int*Freq*Per	**100**	**1**		**1**	95.5650	0.9408	189	101.58	<.0001
Int*Freq*Per	**100**	**1**		**2**	95.6917	0.9231	182	103.66	<.0001
Int*Freq*Per	**100**	**1**		**3**	95.7922	0.9231	182	103.77	<.0001
Int*Freq*Per	**100**	**8**		**1**	94.9033	0.9231	182	102.81	<.0001
Int*Freq*Per	**100**	**8**		**2**	95.5435	0.9359	189	102.09	<.0001
Int*Freq*Per	**100**	**8**		**3**	95.5478	0.9231	182	103.50	<.0001
Int*Freq*Per	**120**	**1**		**1**	95.7873	0.9811	203	97.64	<.0001
Int*Freq*Per	**120**	**1**		**2**	93.0424	0.9359	189	99.41	<.0001
Int*Freq*Per	**120**	**1**		**3**	92.7819	0.9600	196	96.64	<.0001
Int*Freq*Per	**120**	**8**		**1**	95.3662	0.9828	183	97.04	<.0001
Int*Freq*Per	**120**	**8**		**2**	95.6487	0.9828	183	97.33	<.0001
Int*Freq*Per	**120**	**8**		**3**	95.4822	0.9827	183	97.16	<.0001
Week			**1**		96.1529	0.8005	110	120.12	<.0001
Week			**2**		97.1266	0.7975	109	121.79	<.0001
Week			**3**		94.8999	0.7991	110	118.76	<.0001
Week			**4**		94.8613	0.7802	112	121.59	<.0001
Week			**5**		95.8131	0.7718	108	124.14	<.0001
Week			**6**		92.5087	0.7718	108	119.86	<.0001

Least Squares Means									
Effect	**Int**	**Freq**	**Week**	**Per**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Week*Per			**1**	**1**	96.2838	0.9709	189	99.17	<.0001
Week*Per			**1**	**2**	96.1964	0.9673	191	99.45	<.0001
Week*Per			**1**	**3**	95.9787	0.9709	189	98.86	<.0001
Week*Per			**2**	**1**	96.8881	0.9519	182	101.78	<.0001
Week*Per			**2**	**2**	97.1001	0.9805	197	99.03	<.0001
Week*Per			**2**	**3**	97.3917	0.9519	182	102.31	<.0001
Week*Per			**3**	**1**	94.6824	0.9917	197	95.48	<.0001
Week*Per			**3**	**2**	95.0535	0.9520	182	99.85	<.0001
Week*Per			**3**	**3**	94.9638	0.9520	182	99.75	<.0001
Week*Per			**4**	**1**	94.6229	0.9409	189	100.57	<.0001
Week*Per			**4**	**2**	95.1022	0.9231	182	103.02	<.0001
Week*Per			**4**	**3**	94.8589	0.9839	204	96.41	<.0001
Week*Per			**5**	**1**	95.7244	0.9231	182	103.69	<.0001
Week*Per			**5**	**2**	95.7789	0.9231	182	103.75	<.0001
Week*Per			**5**	**3**	95.9360	0.9408	189	101.97	<.0001
Week*Per			**6**	**1**	94.2195	0.9408	189	100.15	<.0001
Week*Per			**6**	**2**	91.7272	0.9231	182	99.36	<.0001
Week*Per			**6**	**3**	91.5794	0.9231	182	99.20	<.0001

C.3 Movement

Table 6-6-9 Model information - SAS® output for Movement using a Randomized 6x6 latin square design

Model Information	
Data Set	WORK.DATA3
Dependent Variable	weight
Covariance Structures	Variance Components, Autoregressive
Subject Effect	Animal(Week)
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Kenward-Roger
Degrees of Freedom Method	Kenward-Roger

Class Level Information		
Class	**Levels**	**Values**
Animal	18	104 125 154 156 161 167 169 178 180 196 240 251 272 279 339 370 392 394
Int	3	80 100 120
Freq	2	1 8
Week	6	1 2 3 4 5 6
Per	3	1 2 3

Covariance Parameter Estimates		
Cov Parm	**Subject**	**Estimate**
Animal		1.2502
AR(1)	**Animal(Week)**	0.2090
Residual		3.2725

Fit Statistics	
-2 Res Log Likelihood	1104.2
AIC (smaller is better)	1110.2
AICC (smaller is better)	1110.3
BIC (smaller is better)	1112.9

Table 6-10 SAS® Type III of fixed effects for movement

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Int	2	73.9	1.91	0.1556
Freq	1	74.4	0.47	0.4934
Int*Freq	2	75.1	0.27	0.7632
Per	2	156	0.15	0.8612
Int*Per	4	160	1.55	0.1910
Freq*Per	2	156	1.85	0.1614
Int*Freq*Per	4	160	1.00	0.4119
Week	5	75.4	3.11	0.0131
Week*Per	10	164	0.43	0.9308

Table 6-11 SAS® Contrast result for movement

Contrasts				
Label	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Linear HR	1	80.9	11.01	0.0014
Quad HR	1	79.4	0.61	0.4387
Cubic HR	1	75.9	0.02	0.8797
Quartic HR	1	72.1	2.53	0.1164

Table 6-12 SAS® least square means results for movement

Least Squares Means									
Effect	**Int**	**Freq**	**Week**	**Per**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Int	**80**				3.5036	0.3439	30.5	10.19	<.0001
Int	**100**				3.1741	0.3404	29.3	9.33	<.0001
Int	**120**				3.7628	0.3430	29.8	10.97	<.0001
Freq		**1**			3.5659	0.3178	22.8	11.22	<.0001
Freq		**8**			3.3944	0.3201	23.2	10.60	<.0001
Int*Freq	**80**	**1**			3.6473	0.4130	53.8	8.83	<.0001
Int*Freq	**80**	**8**			3.3599	0.4026	49.9	8.34	<.0001
Int*Freq	**100**	**1**			3.1308	0.3920	47.2	7.99	<.0001
Int*Freq	**100**	**8**			3.2175	0.4132	52.1	7.79	<.0001
Int*Freq	**120**	**1**			3.9196	0.4046	50.3	9.69	<.0001
Int*Freq	**120**	**8**			3.6059	0.4043	49.9	8.92	<.0001
Per				**1**	3.4040	0.3238	24.5	10.51	<.0001
Per				**2**	3.5290	0.3246	24.7	10.87	<.0001
Per				**3**	3.5074	0.3327	27.1	10.54	<.0001
Int*Per	**80**			**1**	3.1789	0.4171	63.2	7.62	<.0001
Int*Per	**80**			**2**	3.3906	0.4213	65.2	8.05	<.0001
Int*Per	**80**			**3**	3.9412	0.4413	75.6	8.93	<.0001
Int*Per	**100**			**1**	3.2912	0.4144	61.6	7.94	<.0001
Int*Per	**100**			**2**	3.0530	0.4143	61.6	7.37	<.0001
Int*Per	**100**			**3**	3.1783	0.4282	68.3	7.42	<.0001
Int*Per	**120**			**1**	3.7420	0.4193	63.5	8.92	<.0001
Int*Per	**120**			**2**	4.1435	0.4192	63.5	9.88	<.0001
Int*Per	**120**			**3**	3.4028	0.4279	68	7.95	<.0001
Freq*Per		**1**		**1**	3.5339	0.3687	40.7	9.58	<.0001
Freq*Per		**1**		**2**	3.8261	0.3713	41.6	10.31	<.0001

Least Squares Means									
Effect	**Int**	**Freq**	**Week**	**Per**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Freq*Per		**1**		**3**	3.3376	0.3925	50.6	8.50	<.0001
Freq*Per		**8**		**1**	3.2742	0.3771	43.6	8.68	<.0001
Freq*Per		**8**		**2**	3.2319	0.3771	43.6	8.57	<.0001
Freq*Per		**8**		**3**	3.6772	0.3808	45.3	9.66	<.0001
Int*Freq*Per	**80**	**1**		**1**	3.4454	0.5270	125	6.54	<.0001
Int*Freq*Per	**80**	**1**		**2**	3.4355	0.5407	132	6.35	<.0001
Int*Freq*Per	**80**	**1**		**3**	4.0609	0.5834	154	6.96	<.0001
Int*Freq*Per	**80**	**8**		**1**	2.9124	0.5265	125	5.53	<.0001
Int*Freq*Per	**80**	**8**		**2**	3.3457	0.5265	125	6.35	<.0001
Int*Freq*Per	**80**	**8**		**3**	3.8216	0.5399	133	7.08	<.0001
Int*Freq*Per	**100**	**1**		**1**	3.2014	0.5013	111	6.39	<.0001
Int*Freq*Per	**100**	**1**		**2**	3.2472	0.5013	111	6.48	<.0001
Int*Freq*Per	**100**	**1**		**3**	2.9438	0.5488	139	5.36	<.0001
Int*Freq*Per	**100**	**8**		**1**	3.3810	0.5446	132	6.21	<.0001
Int*Freq*Per	**100**	**8**		**2**	2.8588	0.5445	132	5.25	<.0001
Int*Freq*Per	**100**	**8**		**3**	3.4127	0.5446	132	6.27	<.0001
Int*Freq*Per	**120**	**1**		**1**	3.9548	0.5291	125	7.48	<.0001
Int*Freq*Per	**120**	**1**		**2**	4.7958	0.5290	125	9.07	<.0001
Int*Freq*Per	**120**	**1**		**3**	3.0082	0.5426	133	5.54	<.0001
Int*Freq*Per	**120**	**8**		**1**	3.5293	0.5278	124	6.69	<.0001
Int*Freq*Per	**120**	**8**		**2**	3.4911	0.5277	124	6.62	<.0001
Int*Freq*Per	**120**	**8**		**3**	3.7974	0.5412	132	7.02	<.0001
Week			**1**		4.0834	0.4165	58	9.80	<.0001
Week			**2**		3.4472	0.3849	44.2	8.96	<.0001
Week			**3**		4.1144	0.4106	51.7	10.02	<.0001
Week			**4**		3.4269	0.4225	55	8.11	<.0001
Week			**5**		3.0114	0.4223	54.9	7.13	<.0001

Least Squares Means									
Effect	**Int**	**Freq**	**Week**	**Per**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Week			**6**		2.7975	0.3849	44.2	7.27	<.0001
Week*Per			**1**	**1**	4.4540	0.5013	111	8.89	<.0001
Week*Per			**1**	**2**	4.1160	0.5131	118	8.02	<.0001
Week*Per			**1**	**3**	3.6802	0.6790	198	5.42	<.0001
Week*Per			**2**	**1**	3.1724	0.5013	111	6.33	<.0001
Week*Per			**2**	**2**	3.7688	0.5013	111	7.52	<.0001
Week*Per			**2**	**3**	3.4004	0.5013	111	6.78	<.0001
Week*Per			**3**	**1**	4.1274	0.5413	131	7.62	<.0001
Week*Per			**3**	**2**	3.9712	0.5414	131	7.33	<.0001
Week*Per			**3**	**3**	4.2448	0.5415	132	7.84	<.0001
Week*Per			**4**	**1**	3.2521	0.5596	140	5.81	<.0001
Week*Per			**4**	**2**	3.2166	0.5596	140	5.75	<.0001
Week*Per			**4**	**3**	3.8121	0.5598	140	6.81	<.0001
Week*Per			**5**	**1**	2.7610	0.5595	140	4.93	<.0001
Week*Per			**5**	**2**	3.2898	0.5594	140	5.88	<.0001
Week*Per			**5**	**3**	2.9834	0.5596	140	5.33	<.0001
Week*Per			**6**	**1**	2.6574	0.5013	111	5.30	<.0001
Week*Per			**6**	**2**	2.8117	0.5013	111	5.61	<.0001
Week*Per			**6**	**3**	2.9235	0.5013	111	5.83	<.0001

Appendix D. Statistical Results for 20 second period length

D.1 Heart Rate

Table 6-13 Model information - SAS® output for Heart Rate

Model Information	
Data Set	WORK.DATA2
Dependent Variable	HR
Covariance Structures	Variance Components, Autoregressive
Subject Effect	Animal(Week)
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Kenward-Roger
Degrees of Freedom Method	Kenward-Roger

Class Level Information

Class	Levels	Values
Animal	18	104 125 154 156 161 167 169 178 180 196 240 251 272 279 339 370 392 394
Int	3	80 100 120
Freq	2	1 8
Week	6	1 2 3 4 5 6
Per	3	1 2 3

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
Animal		65.7408
AR(1)	**Animal(Week)**	0.7531
Residual		400.53

Fit Statistics	
-2 Res Log Likelihood	2283.3
AIC (smaller is better)	2289.3
AICC (smaller is better)	2289.3
BIC (smaller is better)	2291.9

Table 6-14 SAS® Type III of fixed effects for Heart Rate

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Int	2	77.2	0.22	0.8022
Freq	1	78.3	0.09	0.7624
Int*Freq	2	77.4	1.07	0.3467
Per	2	166	0.38	0.6832
Int*Per	4	166	0.69	0.5978
Freq*Per	2	165	0.26	0.7719
Int*Freq*Per	4	166	2.07	0.0877
Week	5	78.9	2.69	0.0269
Week*Per	10	166	0.77	0.6555

Table 6-15 SAS® Contrast Result for heart rate

Contrasts				
Label	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Linear HR	1	82.1	9.41	0.0029
Quad HR	1	82.8	0.03	0.8712
Cubic HR	1	78	4.01	0.0486
Quartic HR	1	76	0.04	0.8501

Table 6-16 SAS® Least Square Means Results for Heart Rate

Least Squares Means									
Effect	**Int**	**Freq**	**Week**	**Per**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Int	**80**				118.06	3.5552	52	33.21	<.0001
Int	**100**				119.40	3.5773	53.3	33.38	<.0001
Int	**120**				120.87	3.5555	52	34.00	<.0001
Freq		**1**			119.97	3.1334	35.4	38.29	<.0001
Freq		**8**			118.92	3.1003	34	38.36	<.0001
Int*Freq	**80**	**1**			122.02	4.6663	85.6	26.15	<.0001
Int*Freq	**80**	**8**			114.10	4.6362	83.6	24.61	<.0001
Int*Freq	**100**	**1**			117.30	4.7083	88.4	24.91	<.0001
Int*Freq	**100**	**8**			121.50	4.6478	84.3	26.14	<.0001
Int*Freq	**120**	**1**			120.60	4.6693	85.8	25.83	<.0001
Int*Freq	**120**	**8**			121.15	4.6362	83.6	26.13	<.0001
Per				**1**	120.09	2.8842	26.1	41.63	<.0001
Per				**2**	119.68	2.7131	20.8	44.11	<.0001
Per				**3**	118.57	2.7163	20.9	43.65	<.0001
Int*Per	**80**			**1**	119.05	4.0615	84.7	29.31	<.0001
Int*Per	**80**			**2**	119.13	3.8442	70.1	30.99	<.0001
Int*Per	**80**			**3**	116.00	3.8442	70.1	30.17	<.0001
Int*Per	**100**			**1**	121.32	4.2154	95.5	28.78	<.0001
Int*Per	**100**			**2**	118.20	3.8442	70.1	30.75	<.0001
Int*Per	**100**			**3**	118.68	3.8642	71.4	30.71	<.0001
Int*Per	**120**			**1**	119.88	4.0639	84.8	29.50	<.0001
Int*Per	**120**			**2**	121.71	3.8442	70.1	31.66	<.0001
Int*Per	**120**			**3**	121.03	3.8442	70.1	31.48	<.0001
Freq*Per		**1**		**1**	120.29	3.7042	65.2	32.47	<.0001
Freq*Per		**1**		**2**	120.74	3.3271	44.7	36.29	<.0001
Freq*Per		**1**		**3**	118.88	3.3374	45.2	35.62	<.0001

Least Squares Means									
Effect	**Int**	**Freq**	**Week**	**Per**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Freq*Per		**8**		**1**	119.88	3.4537	51.2	34.71	<.0001
Freq*Per		**8**		**2**	118.62	3.3271	44.7	35.65	<.0001
Freq*Per		**8**		**3**	118.25	3.3271	44.7	35.54	<.0001
Int*Freq*Per	**80**	**1**		**1**	118.64	5.5386	151	21.42	<.0001
Int*Freq*Per	**80**	**1**		**2**	123.91	5.0896	118	24.34	<.0001
Int*Freq*Per	**80**	**1**		**3**	123.52	5.0896	118	24.27	<.0001
Int*Freq*Per	**80**	**8**		**1**	119.47	5.3059	134	22.52	<.0001
Int*Freq*Per	**80**	**8**		**2**	114.35	5.0896	118	22.47	<.0001
Int*Freq*Per	**80**	**8**		**3**	108.48	5.0896	118	21.31	<.0001
Int*Freq*Per	**100**	**1**		**1**	121.74	5.7985	170	20.99	<.0001
Int*Freq*Per	**100**	**1**		**2**	116.28	5.0896	118	22.85	<.0001
Int*Freq*Per	**100**	**1**		**3**	113.87	5.1498	122	22.11	<.0001
Int*Freq*Per	**100**	**8**		**1**	120.91	5.3964	141	22.41	<.0001
Int*Freq*Per	**100**	**8**		**2**	120.11	5.0896	118	23.60	<.0001
Int*Freq*Per	**100**	**8**		**3**	123.48	5.0896	118	24.26	<.0001
Int*Freq*Per	**120**	**1**		**1**	120.50	5.5608	153	21.67	<.0001
Int*Freq*Per	**120**	**1**		**2**	122.03	5.0896	118	23.98	<.0001
Int*Freq*Per	**120**	**1**		**3**	119.26	5.0896	118	23.43	<.0001
Int*Freq*Per	**120**	**8**		**1**	119.26	5.3060	134	22.48	<.0001
Int*Freq*Per	**120**	**8**		**2**	121.39	5.0896	118	23.85	<.0001
Int*Freq*Per	**120**	**8**		**3**	122.79	5.0896	118	24.13	<.0001
Week			**1**		130.46	4.8319	96.9	27.00	<.0001
Week			**2**		118.58	4.6166	82.3	25.68	<.0001
Week			**3**		120.67	4.6916	87.3	25.72	<.0001
Week			**4**		119.43	4.6167	82.3	25.87	<.0001
Week			**5**		119.56	4.6092	81.8	25.94	<.0001
Week			**6**		107.97	4.7066	88.3	22.94	<.0001

Least Squares Means									
Effect	**Int**	**Freq**	**Week**	**Per**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Week*Per			**1**	**1**	126.36	6.6953	221	18.87	<.0001
Week*Per			**1**	**2**	131.91	5.0896	118	25.92	<.0001
Week*Per			**1**	**3**	133.10	5.0896	118	26.15	<.0001
Week*Per			**2**	**1**	121.66	5.0896	118	23.90	<.0001
Week*Per			**2**	**2**	117.67	5.0896	118	23.12	<.0001
Week*Per			**2**	**3**	116.39	5.1498	122	22.60	<.0001
Week*Per			**3**	**1**	121.13	5.7271	165	21.15	<.0001
Week*Per			**3**	**2**	121.12	5.0896	118	23.80	<.0001
Week*Per			**3**	**3**	119.77	5.0896	118	23.53	<.0001
Week*Per			**4**	**1**	120.75	5.1505	122	23.45	<.0001
Week*Per			**4**	**2**	118.56	5.0896	118	23.29	<.0001
Week*Per			**4**	**3**	118.99	5.0896	118	23.38	<.0001
Week*Per			**5**	**1**	120.51	5.0896	118	23.68	<.0001
Week*Per			**5**	**2**	118.17	5.0896	118	23.22	<.0001
Week*Per			**5**	**3**	120.00	5.0896	118	23.58	<.0001
Week*Per			**6**	**1**	110.10	5.8370	173	18.86	<.0001
Week*Per			**6**	**2**	110.64	5.0896	118	21.74	<.0001
Week*Per			**6**	**3**	103.15	5.0896	118	20.27	<.0001

D.2 Movement

Table 6-6-17 Model information - SAS® output for Movement

Model Information	
Data Set	WORK.DATA3
Dependent Variable	weight
Covariance Structures	Variance Components, Autoregressive
Subject Effect	Animal(Week)
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Kenward-Roger
Degrees of Freedom Method	Kenward-Roger

Class Level Information

Class	Levels	Values
Animal	18	104 125 154 156 161 167 169 178 180 196 240 251 272 279 339 370 392 394
Int	3	80 100 120
Freq	2	1 8
Week	6	1 2 3 4 5 6
Per	3	1 2 3

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
Animal		0.8746
AR(1)	**Animal(Week)**	0.1701
Residual		4.5880

Fit Statistics

-2 Res Log Likelihood	1200.2
AIC (smaller is better)	1206.2
AICC (smaller is better)	1206.3
BIC (smaller is better)	1208.9

Table 6-18 SAS® Type III of fixed effects for movement

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Int	2	92.1	4.60	0.0125
Freq	1	92.3	0.18	0.6707
Int*Freq	2	93.7	0.59	0.5541
Per	2	172	0.43	0.6489
Int*Per	4	177	3.05	0.0185
Freq*Per	2	172	2.77	0.0652
Int*Freq*Per	4	177	1.14	0.3377
Week	5	92.7	1.08	0.3748
Week*Per	10	181	0.97	0.4724

Table 6-19 SAS® Contrast result for movement

Contrasts				
Label	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Linear HR	1	93.4	6.71	0.0112
Quad HR	1	90.9	2.44	0.1217

Table 6-20 SAS® least square means results for movement

Least Squares Means									
Effect	**Int**	**Freq**	**Week**	**Per**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Int	**80**				2.7540	0.3360	40.1	8.20	<.0001
Int	**100**				2.7469	0.3299	37.5	8.33	<.0001
Int	**120**				3.6749	0.3346	38.6	10.98	<.0001
Freq		**1**			3.1195	0.2991	26.6	10.43	<.0001
Freq		**8**			2.9977	0.3031	27.3	9.89	<.0001
Int*Freq	**80**	**1**			2.6416	0.4277	76.8	6.18	<.0001
Int*Freq	**80**	**8**			2.8665	0.4126	70	6.95	<.0001
Int*Freq	**100**	**1**			2.7741	0.3946	64.8	7.03	<.0001
Int*Freq	**100**	**8**			2.7198	0.4277	73	6.36	<.0001
Int*Freq	**120**	**1**			3.9430	0.4175	71.6	9.44	<.0001
Int*Freq	**120**	**8**			3.4067	0.4139	69.4	8.23	<.0001
Per				**1**	2.9403	0.3142	32.2	9.36	<.0001
Per				**2**	3.1989	0.3140	32	10.19	<.0001
Per				**3**	3.0366	0.3172	33.2	9.57	<.0001
Int*Per	**80**			**1**	2.7882	0.4415	105	6.32	<.0001
Int*Per	**80**			**2**	2.3530	0.4469	108	5.26	<.0001
Int*Per	**80**			**3**	3.1209	0.4638	118	6.73	<.0001
Int*Per	**100**			**1**	2.7405	0.4377	102	6.26	<.0001
Int*Per	**100**			**2**	2.6662	0.4376	101	6.09	<.0001
Int*Per	**100**			**3**	2.8340	0.4417	104	6.42	<.0001
Int*Per	**120**			**1**	3.2923	0.4493	107	7.33	<.0001
Int*Per	**120**			**2**	4.5775	0.4437	104	10.32	<.0001
Int*Per	**120**			**3**	3.1548	0.4437	104	7.11	<.0001
Freq*Per		**1**		**1**	2.7755	0.3797	65.3	7.31	<.0001
Freq*Per		**1**		**2**	3.6329	0.3797	64.9	9.57	<.0001

Least Squares Means									
Effect	**Int**	**Freq**	**Week**	**Per**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Freq*Per		**1**		**3**	2.9503	0.3912	71.4	7.54	<.0001
Freq*Per		**8**		**1**	3.1052	0.3873	68.1	8.02	<.0001
Freq*Per		**8**		**2**	2.7649	0.3872	68.1	7.14	<.0001
Freq*Per		**8**		**3**	3.1229	0.3872	68.2	8.07	<.0001
Int*Freq*Per	**80**	**1**		**1**	2.5748	0.5829	186	4.42	<.0001
Int*Freq*Per	**80**	**1**		**2**	2.1604	0.6003	192	3.60	0.0004
Int*Freq*Per	**80**	**1**		**3**	3.1896	0.6491	209	4.91	<.0001
Int*Freq*Per	**80**	**8**		**1**	3.0016	0.5827	186	5.15	<.0001
Int*Freq*Per	**80**	**8**		**2**	2.5456	0.5826	186	4.37	<.0001
Int*Freq*Per	**80**	**8**		**3**	3.0523	0.5826	186	5.24	<.0001
Int*Freq*Per	**100**	**1**		**1**	2.5339	0.5509	174	4.60	<.0001
Int*Freq*Per	**100**	**1**		**2**	3.1629	0.5509	174	5.74	<.0001
Int*Freq*Per	**100**	**1**		**3**	2.6254	0.5656	181	4.64	<.0001
Int*Freq*Per	**100**	**8**		**1**	2.9472	0.6046	191	4.87	<.0001
Int*Freq*Per	**100**	**8**		**2**	2.1695	0.6043	191	3.59	0.0004
Int*Freq*Per	**100**	**8**		**3**	3.0427	0.6043	191	5.03	<.0001
Int*Freq*Per	**120**	**1**		**1**	3.2178	0.6027	194	5.34	<.0001
Int*Freq*Per	**120**	**1**		**2**	5.5754	0.5855	186	9.52	<.0001
Int*Freq*Per	**120**	**1**		**3**	3.0358	0.5855	186	5.18	<.0001
Int*Freq*Per	**120**	**8**		**1**	3.3668	0.5836	185	5.77	<.0001
Int*Freq*Per	**120**	**8**		**2**	3.5797	0.5835	185	6.13	<.0001
Int*Freq*Per	**120**	**8**		**3**	3.2737	0.5835	185	5.61	<.0001
Week			**1**		3.4342	0.4082	71.3	8.41	<.0001
Week			**2**		2.7641	0.3923	63.6	7.05	<.0001
Week			**3**		3.5361	0.4247	72.9	8.33	<.0001
Week			**4**		3.0368	0.4427	78.3	6.86	<.0001
Week			**5**		2.9286	0.4393	76.5	6.67	<.0001

Least Squares Means									
Effect	**Int**	**Freq**	**Week**	**Per**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Week			**6**		2.6518	0.3923	63.6	6.76	<.0001
Week*Per			**1**	**1**	4.0203	0.5509	174	7.30	<.0001
Week*Per			**1**	**2**	3.2915	0.5659	180	5.82	<.0001
Week*Per			**1**	**3**	2.9909	0.6270	204	4.77	<.0001
Week*Per			**2**	**1**	2.5278	0.5509	174	4.59	<.0001
Week*Per			**2**	**2**	3.2644	0.5509	174	5.93	<.0001
Week*Per			**2**	**3**	2.5001	0.5509	174	4.54	<.0001
Week*Per			**3**	**1**	3.6014	0.6007	191	6.00	<.0001
Week*Per			**3**	**2**	3.1377	0.6009	191	5.22	<.0001
Week*Per			**3**	**3**	3.8692	0.6009	191	6.44	<.0001
Week*Per			**4**	**1**	2.4921	0.6435	205	3.87	0.0001
Week*Per			**4**	**2**	2.9803	0.6232	198	4.78	<.0001
Week*Per			**4**	**3**	3.6379	0.6234	198	5.84	<.0001
Week*Per			**5**	**1**	2.5302	0.6232	198	4.06	<.0001
Week*Per			**5**	**2**	3.6636	0.6230	198	5.88	<.0001
Week*Per			**5**	**3**	2.5921	0.6231	198	4.16	<.0001
Week*Per			**6**	**1**	2.4702	0.5509	174	4.48	<.0001
Week*Per			**6**	**2**	2.8560	0.5509	174	5.18	<.0001
Week*Per			**6**	**3**	2.6293	0.5509	174	4.77	<.0001

Appendix E. Statistical results for mixed procedure for period differences for 1 minute periods

E.1 Heart Rate

Table 6-21 Model information - SAS® output for heart rate P2-P1 differences

Model Information		
Data Set		WORK.DATA3
Dependent Variable		P2_P1
Covariance Structure		Compound Symmetry
Subject Effect		Animal
Estimation Method		REML
Residual Variance Method		Profile
Fixed Effects SE Method		Kenward-Roger
Degrees of Freedom Method		Kenward-Roger
Class Level Information		
Class	**Levels**	**Values**
Animal	18	104 125 154 156 161 167 169 178 180 196 240 251 272 279 339 370 392 394
Int	3	80 100 120
Freq	2	1 8
Week	6	1 2 3 4 5 6
Covariance Parameter Estimates		
Cov Parm	**Subject**	**Estimate**
CS	**Animal**	-7.3164
Residual		145.83

Fit Statistics	
-2 Res Log Likelihood	569.5
AIC (smaller is better)	573.5
AICC (smaller is better)	573.7
BIC (smaller is better)	575.3

Table 6-22 Type III of fixed effects for heart rate P2-P1 differences

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Int	2	57.7	0.98	0.3822
Freq	1	66.8	1.48	0.2287
Int*Freq	2	60.1	0.89	0.4157
Week	5	60.4	0.47	0.7972

Table 6-23 SAS® Least square means results for heart rate P2-P1 differences

Least Squares Means								
Effect	**Int**	**Freq**	**Week**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Int	**80**			-3.7330	2.2429	65.3	-1.66	0.1008
Int	**100**			-4.2281	2.4588	63.4	-1.72	0.0904
Int	**120**			-0.01720	2.2444	64.7	-0.01	0.9939
Freq		**1**		-0.9519	2.0882	42	-0.46	0.6508
Freq		**8**		-4.3670	1.7255	44.5	-2.53	0.0150
Int*Freq	**80**	**1**		0.3697	3.3911	69.9	0.11	0.9135
Int*Freq	**80**	**8**		-7.8356	3.0845	69.3	-2.54	0.0133
Int*Freq	**100**	**1**		-3.1076	3.7230	69.6	-0.83	0.4067
Int*Freq	**100**	**8**		-5.3487	3.2073	69.6	-1.67	0.0999
Int*Freq	**120**	**1**		-0.1177	3.4208	70	-0.03	0.9726
Int*Freq	**120**	**8**		0.08332	3.0811	69.2	0.03	0.9785
Week			**1**	3.0018	4.8050	69.4	0.62	0.5342
Week			**2**	-4.3374	2.7740	68.8	-1.56	0.1225
Week			**3**	-5.3702	3.6387	69.5	-1.48	0.1445
Week			**4**	-4.1556	2.8611	68.9	-1.45	0.1509
Week			**5**	-3.4988	2.7740	68.8	-1.26	0.2115
Week			**6**	-1.5964	3.7817	69.9	-0.42	0.6742

Table 6-24 Model information - SAS® output for heart rate P3-P2 differences

Model Information	
Data Set	WORK.DATA3
Dependent Variable	P3_P2
Covariance Structure	Compound Symmetry
Subject Effect	Animal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Kenward-Roger
Degrees of Freedom Method	Kenward-Roger

Class Level Information

Class	**Levels**	**Values**
Animal	18	104 125 154 156 161 167 169 178 180 196 240 251 272 279 339 370 392 394
Int	3	80 100 120
Freq	2	1 8
Week	6	1 2 3 4 5 6

Covariance Parameter Estimates

Cov Parm	**Subject**	**Estimate**
CS	**Animal**	16.4672
Residual		107.81

Fit Statistics

-2 Res Log Likelihood	762.6
AIC (smaller is better)	766.6
AICC (smaller is better)	766.7
BIC (smaller is better)	768.4

Table 6-25 Type III of fixed effects for heart rate P3-P2 differences

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Int	2	79.4	0.56	0.5724
Freq	1	79.4	0.67	0.4146
Int*Freq	2	79.4	1.46	0.2392
Week	5	79.4	0.36	0.8763

Table 6-26 SAS® Least square means results for heart rate P3-P2 differences

Least Squares Means								
Effect	**Int**	**Freq**	**Week**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Int	**80**			-0.3468	1.9772	57.4	-0.18	0.8614
Int	**100**			0.2130	2.0033	58.9	0.11	0.9157
Int	**120**			-2.2725	1.9772	57.4	-1.15	0.2552
Freq		**1**		-1.6262	1.7197	38	-0.95	0.3503
Freq		**8**		0.02199	1.7062	37.1	0.01	0.9898
Int*Freq	**80**	**1**		1.2050	2.6276	89.7	0.46	0.6476
Int*Freq	**80**	**8**		-1.8986	2.6276	89.7	-0.72	0.4718
Int*Freq	**100**	**1**		-2.2132	2.7053	90.8	-0.82	0.4154
Int*Freq	**100**	**8**		2.6392	2.6276	89.7	1.00	0.3179
Int*Freq	**120**	**1**		-3.8704	2.6276	89.7	-1.47	0.1443
Int*Freq	**120**	**8**		-0.6746	2.6276	89.7	-0.26	0.7980
Week			**1**	-0.4032	2.6276	89.7	-0.15	0.8784
Week			**2**	-0.7641	2.7053	90.8	-0.28	0.7782
Week			**3**	0.4270	2.6276	89.7	0.16	0.8713
Week			**4**	0.9609	2.6276	89.7	0.37	0.7154
Week			**5**	-2.6394	2.6276	89.7	-1.00	0.3178
Week			**6**	-2.3938	2.6276	89.7	-0.91	0.3647

E.2 Eye Temperature

Table 6-27 Model information - SAS® output for Eye Temperature P2-P1 differences

Model Information	
Data Set	WORK.DATA2
Dependent Variable	P2_P1
Covariance Structure	Compound Symmetry
Subject Effect	Animal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Kenward-Roger
Degrees of Freedom Method	Kenward-Roger

Class Level Information		
Class	**Levels**	**Values**
Animal	18	104 125 154 156 161 167 169 178 180 196 240 251 272 279 339 370 392 394
Int	3	80 100 120
Freq	2	1 8
Week	6	1 2 3 4 5 6

Covariance Parameter Estimates		
Cov Parm	**Subject**	**Estimate**
CS	**Animal**	-0.06279
Residual		23.4298

Fit Statistics	
-2 Res Log Likelihood	543.8
AIC (smaller is better)	547.8
AICC (smaller is better)	548.0
BIC (smaller is better)	549.6

Table 6-28 SAS® Type III of fixed effects for eye temperature P2-P1 difference

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Int	2	74.7	1.18	0.3137
Freq	1	74.3	1.85	0.1785
Int*Freq	2	74.2	1.04	0.3588
Week	5	74.3	0.90	0.4872

Table 6-29 SAS® LSM results for eye temperature P2-P1 differences

Least Squares Means								
Effect	**Int**	**Freq**	**Week**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Int	**80**			0.06726	0.8453	76.4	0.08	0.9368
Int	**100**			0.3622	0.8299	75.8	0.44	0.6637
Int	**120**			-1.4047	0.8908	77	-1.58	0.1189
Freq		**1**		-0.9964	0.7014	57.5	-1.42	0.1608
Freq		**8**		0.3463	0.6929	55.3	0.50	0.6192
Int*Freq	**80**	**1**		0.06223	1.1758	85.9	0.05	0.9579
Int*Freq	**80**	**8**		0.07230	1.2198	86	0.06	0.9529
Int*Freq	**100**	**1**		0.03785	1.1756	85.9	0.03	0.9744
Int*Freq	**100**	**8**		0.6866	1.1754	85.9	0.58	0.5607
Int*Freq	**120**	**1**		-3.0894	1.3012	86	-2.37	0.0198
Int*Freq	**120**	**8**		0.2799	1.2141	85.9	0.23	0.8182
Week			**1**	-0.1988	1.2614	86	-0.16	0.8751
Week			**2**	0.1637	1.2560	86	0.13	0.8966
Week			**3**	0.2170	1.2563	86	0.17	0.8633
Week			**4**	0.3948	1.1756	85.9	0.34	0.7378
Week			**5**	0.05427	1.1394	85.9	0.05	0.9621
Week			**6**	-2.5814	1.1756	85.9	-2.20	0.0308

Table 6-30 Model information - SAS® output for Eye Temperature P3-P2 differences

Model Information	
Data Set	WORK.DATA2
Dependent Variable	P3_P2
Covariance Structure	Compound Symmetry
Subject Effect	Animal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Kenward-Roger
Degrees of Freedom Method	Kenward-Roger

Class Level Information

Class	Levels	Values
Animal	18	104 125 154 156 161 167 169 178 180 196 240 251 272 279 339 370 392 394
Int	3	80 100 120
Freq	2	1 8
Week	6	1 2 3 4 5 6

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
CS	**Animal**	0.1164
Residual		1.1350

Fit Statistics

-2 Res Log Likelihood	291.1
AIC (smaller is better)	295.1
AICC (smaller is better)	295.2
BIC (smaller is better)	296.9

Table 6-31 SAS® Type III of fixed effects for eye temperature P3-P2 difference

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Int	2	73.1	0.50	0.6090
Freq	1	72.8	0.01	0.9178
Int*Freq	2	72.1	0.04	0.9602
Week	5	72.5	0.67	0.6504

Table 6-32 SAS® LSM results for eye temperature P3-P2 differences

Least Squares Means								
Effect	**Int**	**Freq**	**Week**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Int	**80**			0.001172	0.2118	65.8	0.01	0.9956
Int	**100**			0.07269	0.1977	60.1	0.37	0.7144
Int	**120**			-0.1863	0.2099	64.4	-0.89	0.3780
Freq		**1**		-0.04883	0.1718	41.8	-0.28	0.7776
Freq		**8**		-0.02616	0.1771	44.3	-0.15	0.8832
Int*Freq	**80**	**1**		-0.04675	0.2718	84.3	-0.17	0.8639
Int*Freq	**80**	**8**		0.04909	0.3030	85.4	0.16	0.8717
Int*Freq	**100**	**1**		0.1002	0.2637	83.9	0.38	0.7048
Int*Freq	**100**	**8**		0.04513	0.2718	84.3	0.17	0.8685
Int*Freq	**120**	**1**		-0.2000	0.2898	85.1	-0.69	0.4920
Int*Freq	**120**	**8**		-0.1727	0.2804	84.7	-0.62	0.5396
Week			**1**	-0.2875	0.2913	85.1	-0.99	0.3265
Week			**2**	0.2509	0.2898	85.1	0.87	0.3891
Week			**3**	-0.09163	0.2718	84.3	-0.34	0.7368
Week			**4**	-0.1737	0.2913	85.1	-0.60	0.5526
Week			**5**	0.2246	0.2718	84.3	0.83	0.4110
Week			**6**	-0.1476	0.2637	83.9	-0.56	0.5771

E.3 Movement

Table 6-33 Model information - SAS® output for Movement P2-P1 differences

Model Information	
Data Set	WORK.DATA1
Dependent Variable	P2_P1
Covariance Structure	Compound Symmetry
Subject Effect	Animal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Kenward-Roger
Degrees of Freedom Method	Kenward-Roger

Class Level Information		
Class	**Levels**	**Values**
Animal	18	104 125 154 156 161 167 169 178 180 196 240 251 272 279 339 370 392 394
Freq	2	1 8
Int	3	80 100 120
Week	6	1 2 3 4 5 6

Covariance Parameter Estimates		
Cov Parm	**Subject**	**Estimate**
CS	**Animal**	0.1912
Residual		6.6056

Fit Statistics	
-2 Res Log Likelihood	432.7
AIC (smaller is better)	436.7
AICC (smaller is better)	436.9
BIC (smaller is better)	438.5

Table 6-34 SAS® Type III of fixed effects for movement P2-P1 difference

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Int	2	68.1	0.53	0.5939
Freq	1	69	0.40	0.5315
Freq*Int	2	69.9	0.59	0.5546
Week	5	69	0.37	0.8672

Table 6-35 SAS® contrast results movement P2-P1 difference

Contrasts				
Label	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Linear 1kHz	1	69.4	0.93	0.3390
Quad 1kHz	1	68.7	0.20	0.6596
Linear 8kHz	1	70	0.24	0.6241
Quad 8kHz	1	68.2	0.82	0.3686

Table 6-36 SAS® least square means results for movement P2-P1 differences

Least Squares Means								
Effect	**Freq**	**Int**	**Week**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Int		**80**		0.1712	0.4777	69.5	0.36	0.7211
Int		**100**		-0.2520	0.4644	67.2	-0.54	0.5892
Int		**120**		0.3960	0.4719	66.9	0.84	0.4043
Freq	**1**			0.2712	0.3846	43.4	0.71	0.4845
Freq	**8**			-0.06103	0.3943	43.8	-0.15	0.8777
Freq*Int	**1**	**80**		-0.06598	0.6775	84.9	-0.10	0.9226
Freq*Int	**1**	**100**		0.04580	0.6145	84.9	0.07	0.9408
Freq*Int	**1**	**120**		0.8338	0.6582	84.9	1.27	0.2087
Freq*Int	**8**	**80**		0.4084	0.6553	85	0.62	0.5348
Freq*Int	**8**	**100**		-0.5498	0.6810	84.9	-0.81	0.4217
Freq*Int	**8**	**120**		-0.04174	0.6548	84.9	-0.06	0.9493
Week			**1**	-0.3958	0.6338	84.9	-0.62	0.5340
Week			**2**	0.5964	0.6145	84.9	0.97	0.3346
Week			**3**	-0.1665	0.6774	85	-0.25	0.8064
Week			**4**	-0.06273	0.7055	85	-0.09	0.9294
Week			**5**	0.5049	0.7052	85	0.72	0.4760
Week			**6**	0.1543	0.6145	84.9	0.25	0.8024

Table 6-37 Model information - SAS® output for movement P3-P2 differences

Model Information		
Data Set		WORK.DATA1
Dependent Variable		P3_P2
Covariance Structure		Compound Symmetry
Subject Effect		Animal
Estimation Method		REML
Residual Variance Method		Profile
Fixed Effects SE Method		Kenward-Roger
Degrees of Freedom Method		Kenward-Roger

Class Level Information		
Class	**Levels**	**Values**
Animal	18	104 125 154 156 161 167 169 178 180 196 240 251 272 279 339 370 392 394
Freq	2	1 8
Int	3	80 100 120
Week	6	1 2 3 4 5 6

Covariance Parameter Estimates		
Cov Parm	**Subject**	**Estimate**
CS	**Animal**	0.2648
Residual		2.5384

Fit Statistics	
-2 Res Log Likelihood	324.5
AIC (smaller is better)	328.5
AICC (smaller is better)	328.7
BIC (smaller is better)	330.3

Table 6-38 SAS® Type III of fixed effects for movement P3-P2 difference

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Int	2	64.2	4.30	0.0177
Freq	1	63.3	4.14	0.0461
Freq*Int	2	65.3	3.62	0.0324
Week	5	64.3	0.69	0.6318

Table 6-39 SAS® contrast results movement P3-P2 difference

Contrasts				
Label	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Linear 1kHz	1	65.6	14.89	0.0003
Quad 1kHz	1	65.3	0.03	0.8624
Linear 8kHz	1	65.2	0.03	0.8632
Quad 8kHz	1	62.8	0.07	0.7872

Table 6-40 SAS® least square means results for movement P3-P2 differences

Least Squares Means								
Effect	**Freq**	**Int**	**Week**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Int		**80**		0.6402	0.3367	60.8	1.90	0.0620
Int		**100**		0.1293	0.3224	56.9	0.40	0.6897
Int		**120**		-0.6102	0.3214	57.1	-1.90	0.0627
Freq	**1**			-0.3020	0.2853	43.4	-1.06	0.2957
Freq	**8**			0.4082	0.2711	38.1	1.51	0.1404
Freq*Int	**1**	**80**		0.8673	0.4778	76.5	1.82	0.0734
Freq*Int	**1**	**100**		-0.2418	0.4451	76.2	-0.54	0.5885
Freq*Int	**1**	**120**		-1.5314	0.4364	76.1	-3.51	0.0008
Freq*Int	**8**	**80**		0.4132	0.4342	76.2	0.95	0.3443
Freq*Int	**8**	**100**		0.5005	0.4368	75.8	1.15	0.2555
Freq*Int	**8**	**120**		0.3111	0.4345	75.9	0.72	0.4762
Week			**1**	0.08473	0.5767	77	0.15	0.8836
Week			**2**	-0.3684	0.3946	75.1	-0.93	0.3536
Week			**3**	0.2790	0.4341	76	0.64	0.5224
Week			**4**	0.5239	0.4521	76.2	1.16	0.2501
Week			**5**	-0.3122	0.4518	76.3	-0.69	0.4916
Week			**6**	0.1118	0.3946	75.1	0.28	0.7777

Appendix F. Statistical results for mixed procedure for period differences for 20 second periods

F.1 Heart Rate

Table 6-41 Model information - SAS® output for heart rate P2-P1 differences

Model Information	
Data Set	WORK.DATA3
Dependent Variable	P2_P1
Covariance Structure	Compound Symmetry
Subject Effect	Animal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Kenward-Roger
Degrees of Freedom Method	Kenward-Roger

Class Level Information		
Class	**Levels**	**Values**
Animal	18	104 125 154 156 161 167 169 178 180 196 240 251 272 279 339 370 392 394
Int	3	80 100 120
Freq	2	1 8
Week	6	1 2 3 4 5 6

Covariance Parameter Estimates		
Cov Parm	**Subject**	**Estimate**
CS	**Animal**	3.9687
Residual		203.21

Fit Statistics	
-2 Res Log Likelihood	598.5
AIC (smaller is better)	602.5
AICC (smaller is better)	602.6
BIC (smaller is better)	604.2

Table 6-42 Type III of fixed effects for heart rate P2-P1 differences

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Int	2	56.7	0.69	0.5082
Freq	1	62.2	0.49	0.4873
Int*Freq	2	61	1.41	0.2531
Week	5	59.3	0.64	0.6692

Table 6-43 SAS® Least square means results for heart rate P2-P1 differences

Least Squares Means								
Effect	**Int**	**Freq**	**Week**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Int	**80**			-0.8695	2.8698	62.2	-0.30	0.7629
Int	**100**			-2.5251	2.9387	60.9	-0.86	0.3936
Int	**120**			1.9817	2.8212	60.2	0.70	0.4851
Freq		**1**		0.6908	2.5745	44.6	0.27	0.7897
Freq		**8**		-1.6327	2.2758	40.1	-0.72	0.4773
Int*Freq	**80**	**1**		3.9458	4.3066	70	0.92	0.3627
Int*Freq	**80**	**8**		-5.6848	3.7694	70	-1.51	0.1360
Int*Freq	**100**	**1**		-4.3054	4.0966	70	-1.05	0.2969
Int*Freq	**100**	**8**		-0.7449	4.0892	70	-0.18	0.8560
Int*Freq	**120**	**1**		2.4318	4.1847	69.9	0.58	0.5630
Int*Freq	**120**	**8**		1.5315	3.7673	70	0.41	0.6856
Week			**1**	6.5096	5.4055	70	1.20	0.2325
Week			**2**	-4.4728	3.5050	70	-1.28	0.2061
Week			**3**	-0.9816	4.2365	69.9	-0.23	0.8175
Week			**4**	-1.9977	3.6362	70	-0.55	0.5845
Week			**5**	-2.3406	3.3926	70	-0.69	0.4925
Week			**6**	0.4573	4.6245	70	0.10	0.9215

Table 6-44 Model information - SAS® output for heart rate P3-P2 differences

Model Information	
Data Set	WORK.DATA3
Dependent Variable	P3_P2
Covariance Structure	Compound Symmetry
Subject Effect	Animal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Kenward-Roger
Degrees of Freedom Method	Kenward-Roger

Class Level Information

Class	**Levels**	**Values**
Animal	18	104 125 154 156 161 167 169 178 180 196 240 251 272 279 339 370 392 394
Int	3	80 100 120
Freq	2	1 8
Week	6	1 2 3 4 5 6

Covariance Parameter Estimates

Cov Parm	**Subject**	**Estimate**
CS	**Animal**	-70.9634
Residual		1949.91

Fit Statistics

-2 Res Log Likelihood	1004.5
AIC (smaller is better)	1008.5
AICC (smaller is better)	1008.6
BIC (smaller is better)	1010.3

Table 6-45 Type III of fixed effects for heart rate P3-P2 differences

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Int	2	70.7	2.13	0.1266
Freq	1	71	1.15	0.2865
Int*Freq	2	70.8	1.18	0.3123
Week	5	70.8	1.02	0.4129

Table 6-46 SAS® Least square means results for heart rate P3-P2 differences

Least Squares Means								
Effect	**Int**	**Freq**	**Week**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Int	**80**			-3.5846	7.2081	83.3	-0.50	0.6203
Int	**100**			16.7355	7.3244	83.8	2.28	0.0248
Int	**120**			-0.6926	7.0867	83	-0.10	0.9224
Freq		**1**		8.7915	5.8128	53.6	1.51	0.1363
Freq		**8**		-0.4860	5.7390	52.7	-0.08	0.9328
Int*Freq	**80**	**1**		-1.3009	10.5510	92.7	-0.12	0.9021
Int*Freq	**80**	**8**		-5.8684	10.2169	92.4	-0.57	0.5671
Int*Freq	**100**	**1**		30.4584	10.5510	92.7	2.89	0.0048
Int*Freq	**100**	**8**		3.0126	10.5501	92.7	0.29	0.7759
Int*Freq	**120**	**1**		-2.7831	10.2169	92.4	-0.27	0.7859
Int*Freq	**120**	**8**		1.3978	10.2169	92.4	0.14	0.8915
Week			**1**	23.0176	10.2169	92.4	2.25	0.0266
Week			**2**	-0.8347	10.9076	93	-0.08	0.9392
Week			**3**	8.3468	10.2169	92.4	0.82	0.4161
Week			**4**	0.04888	10.5501	92.7	0.00	0.9963
Week			**5**	1.8291	10.2169	92.4	0.18	0.8583
Week			**6**	-7.4913	10.2169	92.4	-0.73	0.4653

F.2 Movement

Table 6-47 Model information - SAS® output for Movement P2-P1 differences

Model Information	
Data Set	WORK.DATA1
Dependent Variable	P2_P1
Covariance Structure	Compound Symmetry
Subject Effect	Animal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Kenward-Roger
Degrees of Freedom Method	Kenward-Roger

Class Level Information		
Class	**Levels**	**Values**
Animal	18	104 125 154 156 161 167 169 178 180 196 240 251 272 279 339 370 392 394
Freq	2	1 8
Int	3	80 100 120
Week	6	1 2 3 4 5 6

Covariance Parameter Estimates		
Cov Parm	**Subject**	**Estimate**
CS	**Animal**	-0.1800
Residual		8.3909

Fit Statistics	
-2 Res Log Likelihood	443.5
AIC (smaller is better)	447.5
AICC (smaller is better)	447.7
BIC (smaller is better)	449.3

Table 6-48 SAS® Type III of fixed effects for movement P2-P1 difference

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Int	2	68.5	2.86	0.0639
Freq	1	69.1	3.53	0.0646
Freq*Int	2	70.7	1.03	0.3622
Week	5	69.5	0.99	0.4313

Table 6-49 SAS® contrast results movement P2-P1 difference

Contrasts				
Label	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Linear Int	1	68.9	5.22	0.0254
Quad Int	1	68.1	0.51	.4790
Linear Freq	1	69.1	3.53	0.0646

Table 6-50 SAS® least square means results for movement P2-P1 differences

Least Squares Means								
Effect	**Freq**	**Int**	**Week**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Int		**80**		-0.4693	0.5143	76.2	-0.91	0.3644
Int		**100**		-0.06808	0.4990	75.1	-0.14	0.8919
Int		**120**		1.2264	0.5154	74.9	2.38	0.0199
Freq	**1**			0.7899	0.4093	53.1	1.93	0.0590
Freq	**8**			-0.3306	0.4141	51.5	-0.80	0.4283
Freq*Int	**1**	**80**		-0.4933	0.7445	83.7	-0.66	0.5095
Freq*Int	**1**	**100**		0.6290	0.6754	83.5	0.93	0.3544
Freq*Int	**1**	**120**		2.2340	0.7486	83.7	2.98	0.0037
Freq*Int	**8**	**80**		-0.4453	0.7202	83.7	-0.62	0.5380
Freq*Int	**8**	**100**		-0.7652	0.7483	83.8	-1.02	0.3094
Freq*Int	**8**	**120**		0.2187	0.7193	83.7	0.30	0.7619
Week			**1**	-0.7899	0.6965	83.6	-1.13	0.2600
Week			**2**	0.7366	0.6754	83.5	1.09	0.2786
Week			**3**	-0.4658	0.7442	83.7	-0.63	0.5331
Week			**4**	0.3615	0.8050	83.8	0.45	0.6545
Week			**5**	1.1498	0.7753	83.8	1.48	0.1418
Week			**6**	0.3858	0.6754	83.5	0.57	0.5694

Table 6-51 Model information - SAS® output for movement P3-P2 differences

Model Information	
Data Set	WORK.DATA1
Dependent Variable	P3_P2
Covariance Structure	Compound Symmetry
Subject Effect	Animal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Kenward-Roger
Degrees of Freedom Method	Kenward-Roger

Class Level Information

Class	Levels	Values
Animal	18	104 125 154 156 161 167 169 178 180 196 240 251 272 279 339 370 392 394
Freq	2	1 8
Int	3	80 100 120
Week	6	1 2 3 4 5 6

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
CS	**Animal**	0.9972
Residual		6.9258

Fit Statistics

-2 Res Log Likelihood	428.9
AIC (smaller is better)	432.9
AICC (smaller is better)	433.0
BIC (smaller is better)	434.6

Table 6-52 SAS® Type III of fixed effects for movement P3-P2 difference

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Int	2	67.9	5.44	0.0064
Freq	1	67.4	3.67	0.0596
Freq*Int	2	69	2.01	0.1411
Week	5	68.2	1.07	0.3864

Table 6-53 SAS® contrast results movement P3-P2 difference

Contrasts				
Label	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Linear Int	1	69	10.02	0.0023
Quad Int	1	66.7	0.67	0.4147
Linear Freq	1	67.4	3.67	0.0596

Table 6-54 SAS® least square means results for movement P3-P2 differences

Least Squares Means								
Effect	**Freq**	**Int**	**Week**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Int		**80**		0.7961	0.5579	59.5	1.43	0.1589
Int		**100**		0.1734	0.5272	55.4	0.33	0.7435
Int		**120**		-1.4000	0.5301	54.6	-2.64	0.0108
Freq	**1**			-0.6761	0.4637	39.1	-1.46	0.1528
Freq	**8**			0.3890	0.4584	36.8	0.85	0.4016
Freq*Int	**1**	**80**		1.0455	0.7942	80.8	1.32	0.1917
Freq*Int	**1**	**100**		-0.5832	0.6836	78.7	-0.85	0.3962
Freq*Int	**1**	**120**		-2.4906	0.7087	79.3	-3.51	0.0007
Freq*Int	**8**	**80**		0.5466	0.7050	79.4	0.78	0.4405
Freq*Int	**8**	**100**		0.9299	0.7332	79.5	1.27	0.2084
Freq*Int	**8**	**120**		-0.3095	0.7059	78.9	-0.44	0.6622
Week			**1**	-0.3182	0.7662	80.5	-0.42	0.6791
Week			**2**	-0.7643	0.6634	77.9	-1.15	0.2528
Week			**3**	0.7129	0.7285	79.7	0.98	0.3308
Week			**4**	0.7010	0.7582	80.1	0.92	0.3580
Week			**5**	-0.9658	0.7574	80.3	-1.28	0.2059
Week			**6**	-0.2267	0.6634	77.9	-0.34	0.7335

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Vita

Christina Marie Lyvers

Personal:

Born 1987 in Lebanon, Ky

Education:

University of Kentucky

Bachelors of Science in Biosystems and Agricultural Engineering

Dec 2009

- Courses: Structures and Environment Engineering; Waste Management for Biosystems; Land Treatment of Waste; HVAC;
- Senior Design Project: Design of the Solar Hot Water System for the Sky Blue House

Professional Activities:

Kentucky Student Branch of ASABE Aug '05 - present

- Lawn Mower Clinic Coordinator ('09-'10) – Organized the chapter's annual lawn mower clinic fundraiser that serviced over 200 mowers.
- Secretary ('08-'09)- recorded minutes and coordinated correspondence with other chapters
- Social Chair ('07-'08) – Organized community service and social activities. Organized the BAE Dept. displays for the UK Engineering Day.

ASABE Aug '05 – present

American Society of Agricultural and Biological Engineers

- Attended ASABE International Meeting in 2007, 2010, 2011, and 2012. Presented 2 posters in 2011 and an oral presentation in 2012.
- Attended Midwest and Southern Region Meetings yearly
- Southern Region Constitution Committee Member – 2009
- Served on committee SE- 413 Animal Welfare and Care – 2012
- Help organize and work the Society's booth at the National FFA Convention - 2012

UK Quarter Scale Tractor Team Aug '05 – present

- Graduate Student Advisor ('10-'11 & '11-'12) – Used previous experience and knowledge to advise undergraduate team members during the design process. Helped guide the team to an International Championship in 2012.

- Team Captain (‘08-‘09 & ‘09-‘10) – Coordinated and conducted design meetings and tractor construction. Oversaw the writing of the engineering design report and cost analysis/marketing report.
- Fundraising Chair (‘07-‘08) – Conducted fundraising events and secured sponsors to fulfill the team’s $25,000 Budget.

Alpha Epsilon Spring ‘11 – present

- Inducted Spring 2011
- Kentucky Omega Chapter President (‘11-‘12) – Coordinated meetings and induction ceremonies
- National Vice President (‘11-‘12) – Maintained the website and assisted the president in planning the annual meeting.
- National President (’12-’14) – Organized and led the international meeting

<u>Honors and Awards:</u>

- American FFA Degree - 2007
- BAE Outstanding Junior - 2008
- BAE Outstanding Senior - 2009
- Dean’s List - Spring 2009

<u>Posters:</u>

- Christina M Lyvers, George B Day, Douglas G Overhults. 2011. *Cost-Effective Design for Cattle Squeeze Chutes Which Minimizes Animal Stress*. Poster number SE-06 . ASABE Intl Mtg, Aug 7-11
- Christina M Lyvers, George B Day, Douglas G Overhults. 2011. *Classification of Stress Risers Which May Affect the Design of Squeeze Chutes for Beef Cattle*. Poster number SE-57. ASABE Intl Mtg, Aug 7-11

<u>Presentations:</u>

- Christina M Lyvers, George B Day, Eric Vanzant, and Douglas G Overhults. 2012. *Comparison of Sound Pressure Levels in Handling Facilities to Stress Levels in Beef Cattle.* Paper Number: 121337006. ASABE Intl Mtg, July 2012