EVALUATION OF THE DIVING REFLEX IN RESPONSE TO WATER-BASED FOAM VS. CARBON DIOXIDE GAS DEPOPULATION IN WHITE PEKIN DUCKS

by

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ABSTRACT

The mass depopulation of production birds is an effective means of controlling fast moving, highly infectious diseases such as avian influenza and virulent Newcastle disease. While water-based foam as a method of mass emergency depopulation was conditionally approved by the United States Department of Agriculture Animal and Plant Health Inspection Service in 2006, limited testing has been done with ducks (*Anseriformes).* Three experiments were performed to assess the physiological responses of *Anseriformes* to foam depopulation using White Pekin ducks as a model. Experiment 1 evaluated the difference in time to unconsciousness, motion cessation, brain death, altered terminal cardiac activity, duration of bradycardia, and onset of bradycardia to onset of unconsciousness between foam and CO_2 gas for ducks aged 5-9 weeks. Experiment 2 evaluated the effects of non-terminal submersion in both flowing and standing water and water-based foam on heart rate and duration of bradycardia. Experiment 3 evaluated the effect of abolishing bradycardia on difference in time to unconsciousness, motion cessation, brain death, altered terminal cardiac activity, duration of bradycardia, and onset of bradycardia to onset of unconsciousness between the treatments foam, CO_2 gas and foam with an atropine injection for ducks aged 8-14 weeks. Atropine sulfate was injected to prevent

bradycardia by blocking acetylcholine at parasympathetic neuroeffector sites. Experiment 1 resulted in significantly faster times to all four physiological points for CO_2 gas as opposed to foam. Duration of bradycardia and onset of bradycardia to onset of unconsciousness was significantly longer for foam than CO_2 gas. Experiment 2 determined that both flowing and standing water and foam resulted in a diving reflex that led to pronounced bradycardia. All ducks survived and remained conscious throughout the experiment. Experiment 3 determined that there was a significant correlation between duration of bradycardia and time to unconsciousness, motion cessation, brain death, and altered terminal cardiac activity. The time to unconsciousness, motion cessation, brain death, and altered terminal cardiac activity was significantly faster for the treatment foam with atropine injection as compared to foam. There was no significant difference between foam and CO_2 gas for these physiological points except brain death, in which CO_2 was significantly faster than foam and not significantly different from foam with atropine injection. The results of these three experiments show that bradycardia as a result of the diving reflex occurs as a result of submersion in foam, and that the duration of bradycardia has a significant impact on the time it takes White Pekin ducks to reach unconsciousness and death during foam depopulation. CO_2 gas can also trigger bradycardia and in Experiment 3 resulted in similar time to unconsciousness and death as foam. It can therefore be concluded that both CO_2 gas and water-based foam will trigger bradycardia when

depopulating White Pekin ducks, and extra exposure time should be allowed to compensate for this salvific response.

Chapter 1

INTRODUCTION

The possibility of an outbreak of highly pathogenic avian influenza virus (HPAI), virulent Newcastle disease (VND), or other highly infectious disease is an ongoing concern for the poultry industry. According to the USDA APHIS current interim avian influenza response plan,

> When HPAI outbreaks occur in poultry, the preferred eradication and control methods are quarantine, enforcement of movement restrictions, and depopulation (culling) of all infected, exposed, or potentially infected birds, with proper disposal of carcasses and rigorous cleaning and disinfection of farms and surveillance around affected flocks (USDA, 2007).

Depopulation may also be required for outbreaks of other infectious diseases apart from avian influenza virus (AIV), and may not be limited strictly to gallinaceous birds such as chickens (Benson et al., 2007). The most recent USDA Census of Agriculture (2007) reports that approximately 31.3 million ducks were raised on approximately 31,000 farms in the United States in 2007, which was a 19% increase from 26,000

farms in 2002 (USDA Census of Agriculture, 2009). Disease control needs to be taken into consideration as increasing numbers of ducks, which are also susceptible to reportable diseases such as AIV, are being raised commercially. Compounding the issue is the fact that in many Asian countries where ducks are a major source of protein, ducks often comingle with other livestock such as chickens and swine, while humans often share living spaces and water with these animals (World Organization for Animal Health (OIE), 2004).

The number, variety and widespread distribution of influenza viruses have been far greater in waterfowl, Order Anseriformes, than in other birds (Alexander, 2000). Wild aquatic birds are also the natural reservoir of influenza A viruses (Sturm-Ramirez et al., 2004). In addition, isolations of influenza viruses have been reported from muscovy ducks (*Cairinia moschata*), mallard ducks (*Anas platyrhyncos*), pheasants (*Phasianus* spp.), Japanese quail (*Coturnix coturnix japonica*), chukars (*Alectoris chukar*), guinea fowl (*Numida meleagris*), and various types of goose, all of which can be raised for meat or egg production. When surveillance of commercial ducks from various countries was undertaken, enormous pools of virus and many subtype combinations were detected, especially from meat birds that had been fattened on open fields (Alexander, 2000).

Of notable concern is the HPAI H5N1 that spread across Asia. In a study by Chen et al. (2004), 21 strains of H5N1 influenza viruses isolated from apparently healthy domestic ducks from southern China from 1999 through 2002 were found to be both highly lethal to chickens as well as displayed increasing pathogenicity to mice (Chen et al., 2004). These viruses, however, did not seem to be directly affecting the health of the ducks. This changed in 2002-2003 when new strains were identified that caused mortality in experimentally inoculated domestic ducks (Chen et al., 2004; Ellis et al., 2004; Lee et al., 2005; Sturm-Ramirez et al., 2004; Swayne, 2007). A phylogenetic analysis by Li et al. (2003) showed that emerging H9N2 viruses in southern China that were established in terrestrial poultry had been transmitted back to ducks, suggesting that AIV may be passed back and forth between waterfowl and domestic birds (Li et al., 2003). While reports of these highly pathogenic viruses have been more common in Eurasian countries, there is a risk of an outbreak in the United States that could potentially spread between commercially raised chickens, turkeys, or ducks. These viruses present a human health risk as well, since the transmission of highly pathogenic H5N1 to humans was reported in Hong Kong in 1997 and in southern China in 2003 (Sturm-Ramirez et al., 2004). Since 2003, there have been 596 confirmed human cases of H5N1 avian influenza reported internationally,

resulting in 350 deaths, which is a 59% mortality rate (World Health Organization, 2012).

In the event that a flock needs to be depopulated due to a disease outbreak or a natural disaster, it is important to balance efficiency and the safety of the personnel involved with the welfare of the birds. The American Veterinary Medical Association (AVMA) Guidelines on Euthanasia states that euthanasia techniques should result in rapid loss of consciousness followed by cardiac or respiratory arrest and the ultimate loss of brain function (AVMA, 2007). Mass euthanasia in the event of a disease outbreak such as AI or VND or a natural disaster is considered an unusual circumstance, and accepted options for mass euthanasia include CO_2, gunshot, and penetrating captive bolt (AVMA, 2007). When working with the large numbers of birds that are grown for the food industry, it becomes clear that when culling entire flocks becomes necessary, many of the methods of euthanasia are inadequate and require excessive exposure of personnel to hazardous conditions and the animals to stress.

There are many factors that need to be considered when determining the most appropriate method of mass depopulation. According to the AVMA Guidelines for Euthanasia, some of these considerations include a) animal behavior, or being observant of postures and actions of the animal to ensure the animal in experiencing

minimal distress and b) human behavior, which entails awareness of public perception as well psychological effects of the personnel involved directly or indirectly with the depopulation procedure (AVMA, 2007). The most common methods of mass depopulation attempt to take these considerations into account and to provide the balance between animal welfare, safety, and efficacy. These methods include inhalant agents such as carbon dioxide (CO_2), nitrogen (N_2), and/or argon (Ar) gas and mechanical hypoxia induced by water-based foam (AVMA, 2006; AVMA, 2007).

A large body of research exists in regard to euthanasia and mass depopulation of poultry using inhalant agents, with CO_2 being the most commonly used gas (Alphin et al., 2010; Benson et al., 2007; Gerritzen et al., 2006a; Kingston et al., 2005; McKeegan et al., 2011). CO_2 gas can be applied through whole or partial house gassing, as well as through the use of containerized systems. Whole house gassing involves sealing the structure in which the birds are housed and injecting CO_2 gas into the house through the use of compressed or liquid CO_2. During an outbreak of AI in the Netherlands in 2003, chickens were depopulated using a variety of methods including whole house gassing with CO_2. Times to cessation of motion were not recorded due to the occlusion of the camera lens with fog; however, monitoring of the CO_2 levels indicated that it took between 1800 and 3600 s to reach the desired concentration of 40% at bird level (Gerritzen et al., 2006a). A more recent study of

whole house CO_2 gassing depopulated 28,000 spent laying hens in an 81.7 x 19.2 x 4.6 m, three tiered, caged layer house (McKeegan et al., 2011). Time to death was measured by instrumenting ten laying hens with telemetry equipment to measure electrical brain and cardiac activity, as well as respiratory frequency and body temperature and placing them in cages throughout the house. The average time to death for the instrumented birds was 978 s after the introduction of the liquid CO_2 (McKeegan et al., 2011). A study performed by Kingston et al. (2005) compared the partial house gassing method and a containerized method in the field with CO_2 gas. The partial house method was used to depopulate three flocks of turkeys infected with VND by constructing a covered pen that measured 9.6 x 12.2 x 1.2 m within the poultry house. The times to motion and vocalization cessation were an average of 380 s for the birds that were depopulated using the partial house method. The source of CO_2 for these depopulations was a commercial tanker truck. The containerized method was used to depopulate three flocks of chickens infected with VND, using a container measuring 3 x 1.8 x 1.6 m. The times to cessation of motion and vocalizations were an average of 88 s. The source of CO_2 for these depopulations was a 50-lb gas cylinder (Kingston et al., 2005). The results of the aforementioned experiments show that CO_2 gassing is an effective means of depopulating large numbers of infected or potentially infected poultry in the field.

Depopulation with CO_2 gas, however, is not without its concerns. Each method of delivering CO_2 gas to the poultry has drawbacks, especially when the house cannot be sealed. Ensuring welfare of the birds is a concern with whole house and partial house gassing because large volumes of compressed or liquid CO_2 must be rapidly introduced to the house, which leads to the possibility of freezing temperatures inducing hypothermia in the birds before they lose consciousness (Raj et al., 2006). In addition, gasping and headshaking has been reported in birds exposed to CO_2 gas regardless of application method, which may indicate discomfort and pain (Gerritzen et al., 2004, Gerritzen et al., 2007; McKeegan, 2004; McKeegan et al., 2006, McKeegan et al., 2011; Raj, 1996; Raj et al., 2006). Aside from welfare concerns, mass emergency depopulation through CO_2 gassing introduces logistical challenges including house type, species of bird, method of delivery, personnel requirements, cost, and availability of sufficient quantities of gas during an outbreak situation (Alphin et al., 2010; Gerritzen et al., 2004; McKeegan et al., 2011; Raj et al,. 2006). For these reasons, the mass emergency depopulation method using water-based fire-fighting foam was developed.

Previous studies by Benson et al. (2007, 2009) and Alphin et al. (2010) have extensively compared the effectiveness of CO_2 gassing, argon-carbon dioxide (Ar-CO_2) gassing, water-based foam with ambient air, and water-based foam with CO_2

for the depopulation of floor-reared poultry. These studies included several species of birds such as broilers and layers (*Gallus gallus domesticus)*, turkeys (*Meleagris gallopavo)*, chukars (*Alectoris chukar)*, Japanese quail (*Coturnix japonica)*, call ducks (*Anseriformes platyrhynchos)*, and White Pekin ducks (*Anseriformes domesticus)*. These studies aimed to develop a humane and efficient alternative method of depopulation in accordance to the AVMA Guidelines on Euthanasia (2007).

Over the course of these studies, it was determined that for broilers, foam with ambient air applied in a pre-filled chamber (64 s) and in a pen (274 s) caused cessation of ECG activity significantly faster than CO_2 gas applied under a polyethylene tent (538 s) and in a sealed chamber (139 s) (Benson et al., 2007). Foam with CO_2 gas was not statistically different from foam with ambient air in depopulating broilers, indicating that adding CO_2 gas to foam does not decrease awareness nor does it give foam an anesthetic effect (Alphin et al., 2010). In a study by Benson et al. (2009), foam was tested on chukars, Japanese quail, and ducks. While time to unconsciousness and brain death were not recorded, the times to cessation of motion and cardiac suppression showed that foam can be used to depopulate these species (Benson et al., 2009). As a result of the initial findings of these studies, water-based foam with ambient air as a method of mass depopulation of

floor-reared poultry was conditionally approved by USDA APHIS in 2006, and further studies have been used to support this conditional approval.

A previous study by Benson et al. (2009) showed that foam can be successfully used to depopulate ducks, however, the time it took for the animals to reach unconsciousness and brain death were not recorded as they were in other studies on layers, broilers, and turkeys. In addition, only a limited number of birds were used in the study. The results of this study did show, however, that while a 100% mortality rate was achieved using water-based foam, it took longer for White Pekin ducks to reach motion cessation as determined by accelerometer (352.0 s) than chukars (165.3 s) and quail (50.3 s) depopulated in a similar manner. The longer times for ducks may be due to the diving reflex, which causes changes in heart rate such as bradycardia. The diving reflex is observed in ducks subjected to submersion, which allow them to conserve oxygen while diving (Bamford & Jones, 1974; Bryan & Jones, 1980; Butler & Taylor, 1973; Jones et al., 1988; Smith & Jones, 1990; Stephenson et al., 1994).

There have been several publications describing the mechanisms by which ducks respond to hypoxia and hypercapnia. Butler and Taylor (1973) found that neither hypoxia nor hypercapnia alone or together had any significant effect on heart rate in spontaneously breathing ducks (Butler & Taylor, 1973). During submersion, however, pronounced bradycardia occurred. Cessation of artificial ventilation in

paralyzed ducks caused bradycardia similar to ducks undergoing forced submersion. These results implied that having water in contact with the beak, nares, or internal respiratory passages is not necessary to elicit a diving-type response. The fact that water may not be necessary for eliciting a diving response or bradycardia has implications for foam depopulation. The foam used during mass emergency depopulation is a mixture of water, air, and foam concentrate. For depopulation, the ratio of liquid to the foam produced ranges from a low of 1:25 to a high of 1:140, resulting in relatively low water concentration in the foam. During foam depopulation, water itself is not coming into contact with the beak, nares, or respiratory passages, but rather the bubbles from the foam contact these areas. The foam bubbles then lead to occlusion of the trachea which leads to mechanical hypoxia (Benson et al., 2007).

Bamford and Jones (1974) determined that stimulation of the glottal receptors in White Pekin and Khaki Campbell ducks produced apnea (Bamford & Jones, 1974). The longest periods of apnea were produced when cold water was poured over the nares, glottis, and upper beak, while the period of apnea was shorter when warm water, oil, or air was used. No significant changes in heart rate were caused by stimulation of the glottal and trigeminal receptors, which suggested that the cardiovascular adjustments observed were in response to the apnea. It is possible that the action of the foam flowing over the beak, nares, or glottis stimulates apnea in the

duck, which would lead to cardiovascular adjustments. It is also possible that apnea produced by the occlusion of the trachea may cause cardiac adjustments as well.

In addition to apnea produced by the stimulation of glottal receptors, studies have shown that in dabbling ducks, such as the Pekin and Mallard, arterial chemoreceptor stimulation and baroreceptors play a role in the cardiovascular adjustments seen during diving (Jones et al., 1988; Smith & Jones, 1990; Smith & Jones, 1992). There are also differences between the cardiac adjustments observed during a forced dive and a voluntary dive, such as when a dabbler is trained to dive for food. During a restrained forced dive, or when access to the surface is denied during a voluntary dive, bradycardia occurs and blood flow is directed to the brain and central cardiovascular areas. In contrast, heart rate during a voluntary dive is generally above the resting rate and blood flow is directed to the muscles of the hind limbs (Jones et al., 1988).

Bryan and Jones (1980) found that oxygen conserving cardiovascular adjustments played a key role in increased tolerance to asphyxia. Using mallard ducks (*Anas platyrhynchos*), tolerance to asphyxia was tested by restrained submergence in water and by stopping artificial ventilation in paralyzed ducks. Some ducks were treated with atropine sulfate (2.5 mg/kg) to prevent cardiovascular adjustments during apneic asphyxia. Atropine sulfate works by blocking acetylcholine at postganglionic

parasympathetic neuroeffector sites, which at moderate doses increases the heart rate and prevents bradycardia (Veterinary Drug Handbook, 2002). These ducks showed a significant decrease in tissue dissolved oxygen (pO_2) and an increase in nicotinamide adenine dinucleotide (NADH) fluorescence during asphyxia, which compared to non-atropinized ducks, indicates that oxygen was used up more quickly in the respiratory chain leading to the accumulation of reduced NAD as detected by its natural fluorescence. The results of this study indicated that cardiovascular adjustments during asphyxia were critical to conserving oxygen.

Ducks have physiological differences from gallinaceous birds, which some studies suggest makes them more difficult to euthanize (Gerritzen et al., 2007; Raj, 2008) while others found that there was no significant difference in their ability to withstand hypercapnia and hypoxia (Gerritzen et al., 2006b). Due to these differences, it is important to closely examine the physiological responses of ducks to depopulation using water-based foam to ensure the welfare of the animal. The author hypothesized that water-based foam would trigger the diving reflex and result in bradycardia during the depopulation of ducks and that bradycardia triggered by foam depopulation has a salvific effect by prolonging time to unconsciousness and death as compared to CO_2 gassing. The objective of the first experiment was to determine if there was a significant difference in time to death, including unconsciousness and brain death,

between depopulation using foam or CO_2 gas. The objective of the second experiment was to determine if submersion of the bill in foam lead to apnea and bradycardia similar to submersion in water, and if the foam needed to be flowing across the bill to trigger these responses. The objective of the third experiment was to determine if bradycardia as a result of the diving reflex was responsible for the prolonged time until death. The project was performed in three experiments as described below.

Chapter 2

MATERIALS AND METHODS

General Procedure and Instrumentation

Approximately 24 - 48 h before a trial, 4 ducks were randomly selected for surgery. Ducks selected for surgery had food withheld for approximately 8 h and water withheld for approximately 2-6 h before surgery since waterfowl take in a large quantity of water and can regurgitate under anesthesia (Avian Medicine, 2009). Each duck was anesthetized using 5% isoflurane (IsoSol; Vedco, Inc., St. Joseph, MO) at induction with 3% isoflurane for maintenance of anesthesia. One Data Sciences International (DSI; St. Paul, MN) three-channel PhysioTel Model F50-EEE wireless transmitter per duck was surgically implanted in the back of the neck of each duck. Three leads were placed on the meninges covering the telencephalon through 0.9 mm holes that were drilled into the parietal bone, two holes on the right side of the midline and one on the left, using a Fine Science Tools (Foster City, CA) Model 18000-17 high-speed microdrill. Two leads were implanted in the complexus muscle just below the skull for electromyography (EMG). The ducks were injected subcutaneously with

0.4 mg/kg carprofen after the procedure and were allowed to recover for 24 h. The surgical procedure is based on Savory and Kostal (1997, 2006) and has been used by the research group with broilers (Alphin et al., 2010), turkeys (Rankin, 2010), layers, and ducks.

Signals from the wireless transmitter were recorded by 2 DSI RMC-1 PhysioTel receivers placed opposite one another on either side of the animal's head and the signals from the receivers were passed through a DSI Matrix. Brain activity was monitored and recorded using DSI Dataquest A.R.T. Acquisition software. Electroencephalogram (EEG) files were analyzed in DSI NeuroScore software.

The raw EEG signal was analyzed in NeuroScore by adding labeled markers over 2 s epochs indicating pre-treatment (first 420 s), treatment (60 s), and post-treatment periods (420 s). The markers were placed based on visual analysis of the EEG signal using the EMG signal as a reference to eliminate motion artifacts, which appear as high amplitude spikes in both the EEG and EMG channels. The mean EEG, mean EMG, alpha (8-12 Hz), beta (16-24 Hz), delta (0.5-4 Hz), theta (4-8 Hz), and sigma (12-16 Hz) values and markers were exported on a 2 s basis from Neuroscore to Excel (Microsoft Corp., Redmond, WA) and charted.

For the depopulation experiments, signals from the wireless transmitter were recorded by 2 DSI RMC-1 PhysioTel receivers placed opposite one another at the bottom of a 208-L chamber. EEG files were analyzed in DSI NeuroScore software to detect EEG silence (brain death) and unconsciousness. EEG silence was determined to be the point at which the mean signal over 1 s periods is stable (minimal to no change) about 0 mV, which is an indication of brain death. Unconsciousness was determined using the relative power band ratio alpha/delta, which monitors a trend from high frequency brain wave activity to low frequency brain activity (Benson et al., 2012). The raw EEG signal was analyzed in NeuroScore by marking 2 s epochs as described above indicating pre-treatment (first 60 s), post-treatment, convulsion, and post-convulsion periods based on visual analysis. Values and markers were exported from Neuroscore to Excel (Microsoft Corp, Redmond, WA) and charted as described above.

A PCB Piezotronics shear mode accelerometer was attached to the left leg to measure the time of motion cessation. Two different accelerometers were used depending on availability. The model 353B16 accelerometer has a sensitivity of 1.02 $mV \cdot s^2/m$ ± 10% (10 mV/g ± 10%) capable of operating over a range of ±4,905 m/s^2 (500 g) of peak. The higher sensitivity model 352C66 accelerometer operates at 10.2 $mV \cdot s^2/m$ ± 10% (100mV/g ± 10%) over a range of ±491 m/s^2 (± 50 g) of peak. For

depopulation, the differing sensitivities and operational ranges are inconsequential, as the signal characteristic of interest is a mean 0 V signal (flat line) occurring after convulsions. The output from the accelerometer was passed through a PCB Piezotronics model 480C02 single-channel signal conditioner connected to a National Instruments PCI-6036E data acquisition card (Austin, TX). The conditioned signal was collected at 100 Hz in a custom written National Instruments (Austin, TX) LabVIEW virtual instrument. Text files generated by the virtual instrument was processed through a custom program written in Visual Basic for Applications in Excel (Microsoft Corp., Redmond, WA) to reduce the signal frequency and chart the data (Benson et al., 2009). The use of an accelerometer to monitor poultry during depopulation was validated by Dawson et al. (2009) and Dawson et al. (2007).

To measure electrical cardiac activity, each duck was instrumented with electrocardiogram (ECG) electrodes and leads (BIOPAC Systems Inc., Goleta, CA) placed on a previously plucked area on each leg and underneath the right wing. ECG signals were processed through BIOPAC Systems, Inc. MP30A acquisition unit and recorded using BIOPAC Student Lab (BSL) software. Analysis of the ECG signals was conducted using BIOPAC BSL Pro to analyze the recorded signals and find critical points. Critical points included onset and duration of bradycardia and onset of altered terminal cardiac activity (ATCA). Bradycardia was defined for analysis

purposes in this study as a heart rate that dropped to 100 beats per minute (bpm) or less and sustained the lowered rate for at least 10 consecutive seconds, followed by a return to a rate in excess of 100 bpm. ATCA is defined in this study as the irreversible cessation of rhythmic electrical cardiac activity that invariably results in an isoelectric ECG. The point of ATCA must be selected after evidence of motion artifact, and therefore terminal convulsions, ceases and arrhythmic electric activity begins (M. K. Rankin, 2010, University of Delaware, Newark, Delaware, personal communication). All testing was performed under the approval and guidelines of the University of Delaware Agricultural Animal Care and Use Committee and followed the guidelines laid out by the Federation of Animal Science Societies (Federation for Animal Science Societies, 1999).

Foam and CO_2 Gas Treatments

For the foam treatments, water based foam with ambient air was created using a nozzle type foam depopulation system (model AG-1, Spumifer, Ridgefield Park, NJ). A 1% solution of foam concentrate (model MD-881, Phos-Check, St. Louis, MO) and water was premixed on the day of trial. A gasoline pump (model 2 1/2AGE 31 BS, Darley, Itasca, IL) was used to supply the required pressure and flow. The pump was driven by a 23-kW (31 hp) Briggs & Stratton (Milwaukee, WI)

Vanguard gasoline engine providing a rated performance of 1136 L/min at 586 kPa (Benson et al. 2009, Alphin et al. 2010). For the CO_2 gas treatments, 100% CO_2 was applied into the sealed chamber at a rate of 2265 L/min until the end of terminal convulsions.

Experiment 1

Experiment 1 aimed to test the hypothesis that water-based foam and CO_2 gas as methods of depopulation would result in similar times to unconsciousness and death. To evaluate the use of water based foam as a method of depopulation for ducks, 46 straight run White Pekin ducks from a commercial hatchery were individually depopulated using either water-based foam or 100% CO_2 gas.

Birds were raised to between 5 and 9 weeks of age following standard care and conditions. Each duck underwent surgery to implant the wireless EEG transmitter as described above. Each bird was instrumented with an accelerometer, ECG leads and a surgically implanted EEG transmitter as described above. Each duck was placed individually in a 208 L chamber, a 60 s pretreatment baseline was recorded, and either one of two treatments (foam or CO_2 gas) was applied. Treatment order was determined randomly using an Excel random table. Carbon dioxide gas was applied as

described above. Foam was applied until the chamber was full, which typically required less than 5 s. Sensor data was recorded for a total of 900 s (15 min).

For data analysis, critical times for physiological events were extracted from the EEG, ECG, and accelerometer data as described above, compiled in Excel and statistical analysis was performed using SAS. The SAS data set was coded to extract sensor data valid for treatment analysis. The extracted treatment data was used to determine the distribution of analysis-specific data sets. The data subsets were not normally distributed and thus a non-parametric statistical analysis was conducted on each data subset in SAS. A Wilcoxon Exact test and Student's T-test was used to analyze the treatment-dependent data sets. All tests were conducted at the 5% (α = 0.05) significance level.

Experiment 2

The aim of Experiment 2 was to test the hypothesis that non-terminal submersion of ducks into water-based foam would trigger the diving reflex and result in bradycardia. For this experiment, 8 straight run White Pekin ducks from the same commercial hatchery, 8-11 weeks old and 2-3.2 kg were fitted with telemetry equipment to measure brain activity (electroencephalogram), electrical cardiac activity and heart rate (electrocardiogram) as described above.

The ducks were restrained horizontally, ventral side down in a wooden cradle and immobilized using a full body length, custom restraint jacket. A hood was placed over the ducks' eyes to prevent anticipation of any treatment and reduce movement. A baseline of 420 s was established. After this point, the duck was exposed to one of four random treatments as determined by an Excel random table: (1) water dunk (WD), (2) foam dunk (FD), (3) water pour (WP), or (4) foam pour (FP). The bill was submerged by lowering the head into a container of either water (1) or foam (2) so that the nares were covered. Water (3) or foam (4) was poured over the bill, flowing over the nares for the full 60 s treatment period.

For the foam pour and water pour treatments, foam or water respectively was poured into a 208 L drum situated on a platform above the duck using the hose from the foam system. The 208 L drum was fitted with a sliding lever that allowed the flow rate of the foam or water to be adjusted to achieve continuous flow over the 60 s treatment period.

The ducks were given at least one hour between tests to recover (Hudson & Jones, 1986). The ducks were closely monitored and submerged for a maximum of 60 s and were immediately removed if respiration continued while submerged. During the course of this experiment, however, no ducks attempted respiration while submerged. After 60 s of submersion, the ducks were allowed to recover for an

additional 420 s, for a total recording time of 900 s. There were 2 groups of 4 ducks, and each duck was exposed to each treatment at least once, with a total of 65 trials completed.

Statistical analysis was performed using the Wilcoxon Two-Sample Test, Kruskal-Wallis Test, Student's T-Test and Spearman Correlation in SAS. All tests were conducted at the 5% ($\alpha = 0.05$) significance level

Experiment 3

Experiment 3 aimed to test the hypothesis that bradycardia triggered by foam depopulation has a salvific effect by prolonging time to unconsciousness and death as compared to CO_2 gassing. The third experiment consisted of 64 straight run White Pekin ducks 8-14 weeks of age from the same commercial hatchery as the two previous experiments. The ducks were fitted with ECG leads, EEG implant, and accelerometer as described above. An age of 8 weeks was selected because this is the typical market age of a Pekin duck; depopulation at this age represents a worst-case scenario for the industry. In addition, according to the growth curve appearing in Gille and Salomon (2000), gains in body mass should slow down after this point, which will help reduce variations in dive time according to Hudson and Jones (1986).

For the foam + atropine treatment, atropine sulfate was injected at the lowest effective dose, which was determined to be 0.5 mg/kg atropine sulfate (0.54 mg/ml) intramuscularly (IM) into the breast muscle 15 minutes before depopulation by foam. The purpose of the IM atropine injection was to prevent bradycardia as described by Butler and Jones (1971) and Bryan and Jones (1980). Heart rate was monitored during the 60 s baseline to ensure that the heart rate was stable before treatment with water-based foam. Pre-experiment testing was performed to determine dosing and time requirements.

Approximately 24 - 48 h before a trial surgery was performed as described above. Each duck was placed individually in a 208 L chamber and one of three random treatments, as determined by an Excel random table, of foam, CO_2 gas, or foam + atropine was applied. The foam treatment consisted of 21 ducks (n = 21), the foam + atropine injection treatment consisted of 20 ducks (n = 20), and the CO_2 gas treatment consisted of 23 ducks (n = 23). Carbon dioxide gas was applied as described above. Foam was applied until the chamber was full for both the foam and foam + atropine injection treatments. The depopulation trial was recorded for 900 s (15 min).

Data was extracted from the sensors as described above. Statistical analysis was performed in SAS using the Wilcoxon Two-Sample Test, Kruskal-Wallis Test, and Spearman Correlation in SAS because the data was not normally distributed.

Additional analysis was performed using Tukey's Studentized Range to determine difference of means. All tests were conducted at the 5% ($\alpha = 0.05$) significance level.

Chapter 3

RESULTS

Experiment 1: Depopulation

The first experiment resulted in 1 survivor for the CO_2 gas treatment (not included in the analysis) and no survivors for the foam treatment. CO_2 gas was faster than water based foam for all 4 physiological points measured. In Figure 1, the average time to each of the 4 physiological points for each treatment is shown. Unconsciousness was the first physiological condition reached, followed by motion cessation, brain death, and finally ATCA. As seen in Table 1, CO_2 caused the ducks to reach unconsciousness faster ($X = 77$ s) than foam ($X = 142$ s) ($p < 0.001$). The time to motion cessation was faster for CO_2 ($X = 168$ s) than for foam ($X = 243$ s) ($p < 0.001$). The time to brain death was faster for CO_2 ($X = 171$ s) than for foam ($X = 283$ s) ($p < 0.001$). Similarly, the time to ATCA was faster for CO_2 ($X = 234$ s) than for foam ($X = 300$ s) ($p = 0.003$).

Table 1 **Time in seconds to the onset of important physiological conditions in 5-9 week old White Pekin ducks depopulated with water-based foam or CO_2 gas. Data presented as mean ± standard error of the mean (X ± SEM). Means within a row with different superscripts differ ($p < 0.05$)**

	Treatment	
Variable	Water-Based Foam	CO_2 Gas
Unconsciousness	142 ± 8^a	77 ± 11^b
Motion Cessation	243 ± 16^a	168 ± 10^b
Brain Death	283 ± 14^a	171 ± 8^b
ATCA	300 ± 18^a	234 ± 9^b
Duration of Bradycardia	176 ± 19^a	79 ± 8^b
Onset of Bradycardia to Onset of Unconsciousness	114 ± 10^a	37 ± 7^b

The variable duration of bradycardia and onset of bradycardia to onset unconsciousness were also analyzed as described in Materials and Methods. The foam treatment generated a longer duration of bradycardia than the CO_2 treatment ($p <$

0.001). The time from the onset of bradycardia to onset of unconsciousness was longer for the foam treatment compared to the CO_2 treatment ($p < 0.001$).

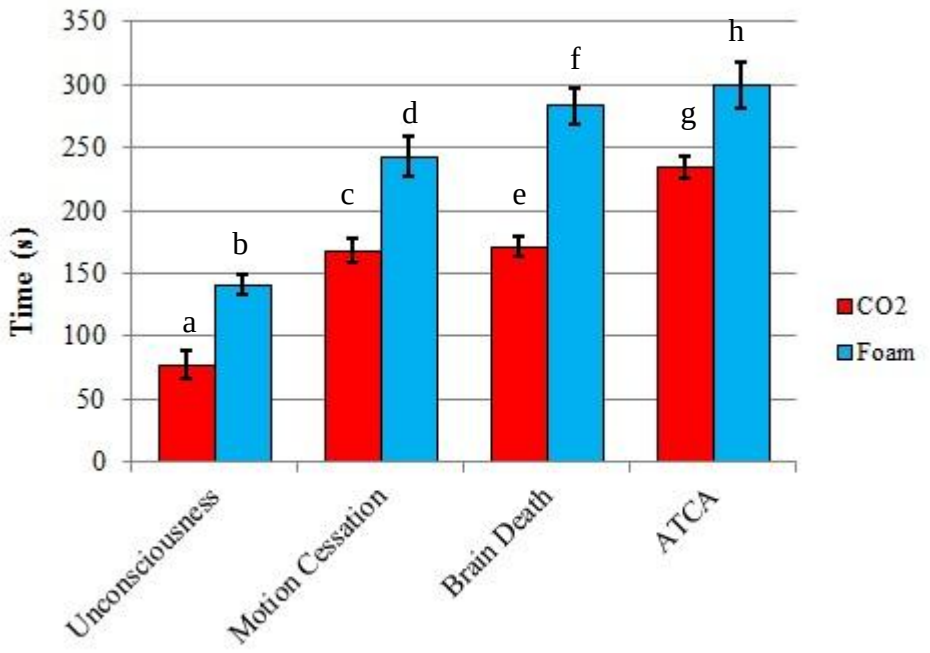

Figure 1 **Comparison of mean time (seconds) for White Pekin ducks to reach four physiological points after individual depopulation with either CO_2 gas or water-based foam. Data presented as mean ± standard error of the mean (X ± SEM). Letters denote statistical significance between treatments within physiologic variables ($\alpha = 0.05$).**

Figures 2 and 3 show representative ECG charts from two individual ducks taken between 230 s and 258 s of the 900 s recording. This particular period of time showed differences in heart rate by treatment for these two individuals since duck 20100810 D3 did not display bradycardia, however, the onset and duration of bradycardia typically varied between ducks (Table 1).

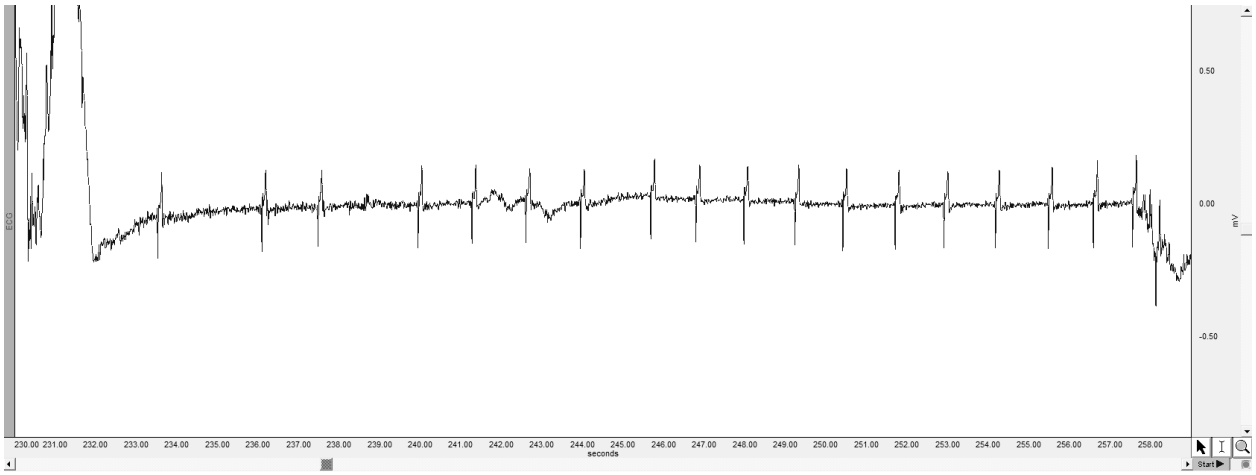

Figure 2 **Representative ECG recording for an individual duck (Duck 20100805 D3) depopulated with water based foam, showing period from 230 s to 258 s out of a 900 s recording.**

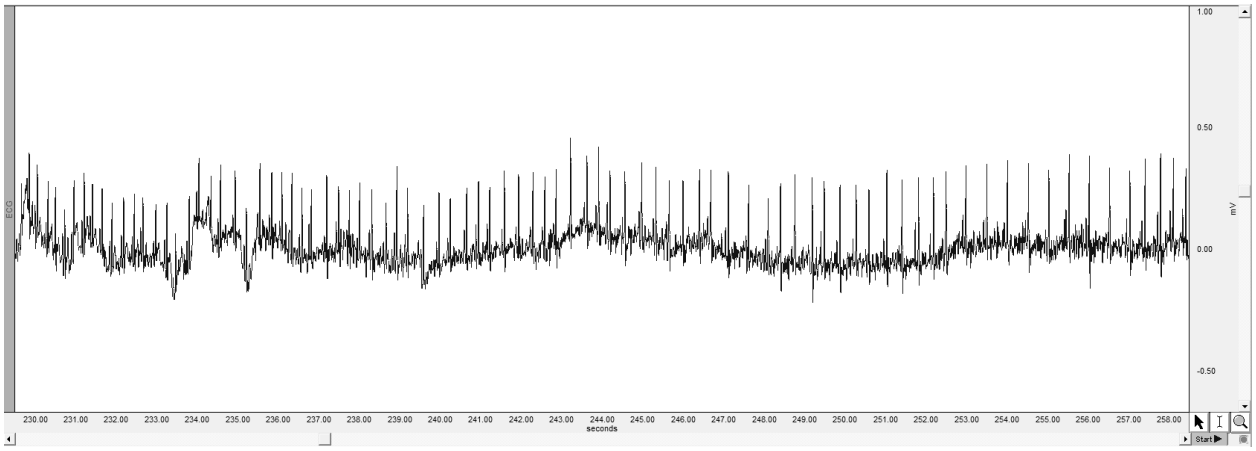

Figure 3 Representative ECG recording for an individual duck (20100810 D3) depopulated with CO_2 gas, showing period from 230 s to 258 s out of a 900 s recording.

The duck depopulated with foam (Duck 20100805 D3, Fig. 2) developed a bradycardia of 46 bpm as compared to the 166 bpm heart rate recorded for a duck depopulated with CO_2 gas (Duck 20100810 D3, Fig. 3) during the same period of time. Bradycardia was seen in 18 out of 19 or 95% of ducks treated with foam, as opposed to 9 out of 18 or 50% of ducks treated with CO_2.

Experiment 2: Submersion

The 900 s recording period for this experiment was broken into 8 critical time periods for analysis. The time periods are based on relation to the treatment period: the first 60 s of the pre-treatment (0-30 s and 30-60 s), the last 30 s of the pre-treatment (390-420 s), the treatment (420-450 s and 450-480 s), the first 30 s of post-treatment (480-510 s), and the last 60 s of the post-treatment period (840-870 s and 870-900 s). Figure 4 below shows that all four treatments caused a drop in heart rate that met the definition of bradycardia as defined in Materials and Methods. As seen in Table 2, no differences occurred in heart rate between treatment groups during the pre-treatment periods (0-30 s, 30-60 s, and 390-420 s) ($p > 0.05$). Additionally, no differences in heart rate occurred across treatment groups for FP and WP or FD and WD at any point during the trial period.

Table 2 **Mean heart rate of White Pekin ducks in beats per minute (bpm) for the treatments foam dunk (FD), foam pour (FP), water dunk (WD), and water pour (WP). Time periods are in 30 seconds intervals of a 900 second trial. Data presented as mean ± standard error of the mean (X ± SEM). Means within a row with different superscripts differ ($p < 0.05$)**

	Treatment			
Time Period	FP	FD	WD	WP
0-30	183.5 ± 10.8^{a}	190.6 ± 11.6^{a}	175.1 ± 6.4^{a}	182.1 ± 5.0^{a}
30-60	185.1 ± 10.9^{a}	181.7 ± 6.8^{a}	172.0 ± 5.5^{a}	179.9 ± 6.0^{a}
390-420	191.5 ± 6.7^{a}	194.0 ± 8.4^{a}	195.0 ± 7.5^{a}	189.5 ± 5.8^{a}
420-450	139.9 ± 7.1^{a}	117.8 ± 10.1^{b}	1117.1 ± 8.4^{b}	153.9 ± 6.0^{a}
450-480	51.1 ± 5.6^{a}	56.6 ± 4.6^{a}	56.6 ± 4.1^{a}	58.6 ± 7.5^{a}
480-510	85.5 ± 15.7^{a}	200.1 ± 17.3^{b}	170.4 ± 14.1^{bc}	135.0 ± 18.6^{ac}
840-870	198.9 ± 13.0^{a}	173.6 ± 8.2^{ab}	161.9 ± 6.9^{bc}	174.9 ± 9.1^{ac}
870-900	199.2 ± 12.5^{a}	179.5 ± 8.9^{ab}	163.9 ± 5.4^{b}	174.0 ± 9.0^{ab}

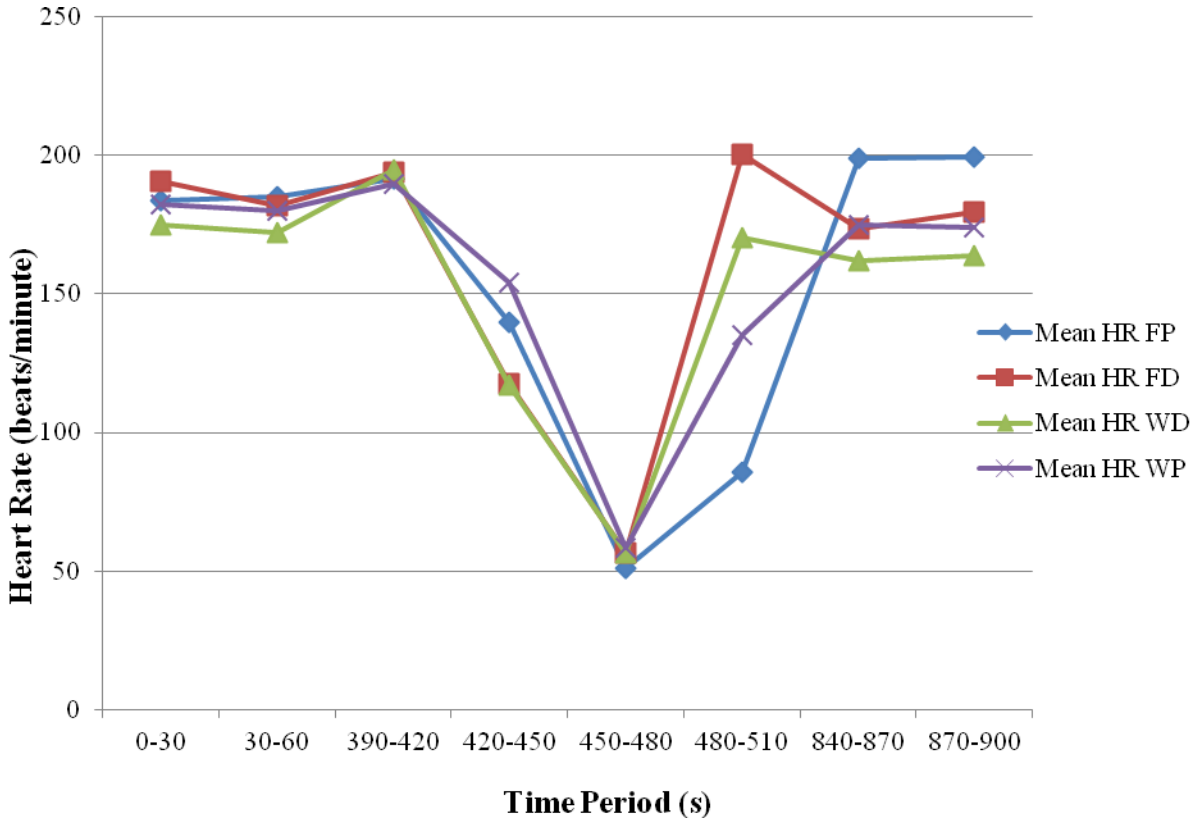

Figure 4 **The line graph above shows the mean heart rate (HR) of White Pekin ducks that had foam (FP) or water (WP) poured on their bills or had their bills dipped in foam (FD) or water (WD). For analysis purposes, the trial was broken into 8 critical periods over the 900 s trial.**

There was an effect of different treatments on heart rate were found between treatments during the treatment periods. Differences occurred between FP and FD ($p = 0.032$), FP and WD ($p = 0.013$), FD and WP ($p = 0.010$), and WP and WD ($p = 0.002$) during the first 30 s of treatment (420-450 s). During the first 30 s of the post-treatment (480-510 s), differences in heart rate occurred between FP and FD ($p < 0.001$), FP and WD ($p = 0.003$), and FD and WP ($p = 0.011$). Differences occurred between FP and WD during the 840-870 s period ($p = 0.015$) and the 870-900 s period ($p = 0.015$).

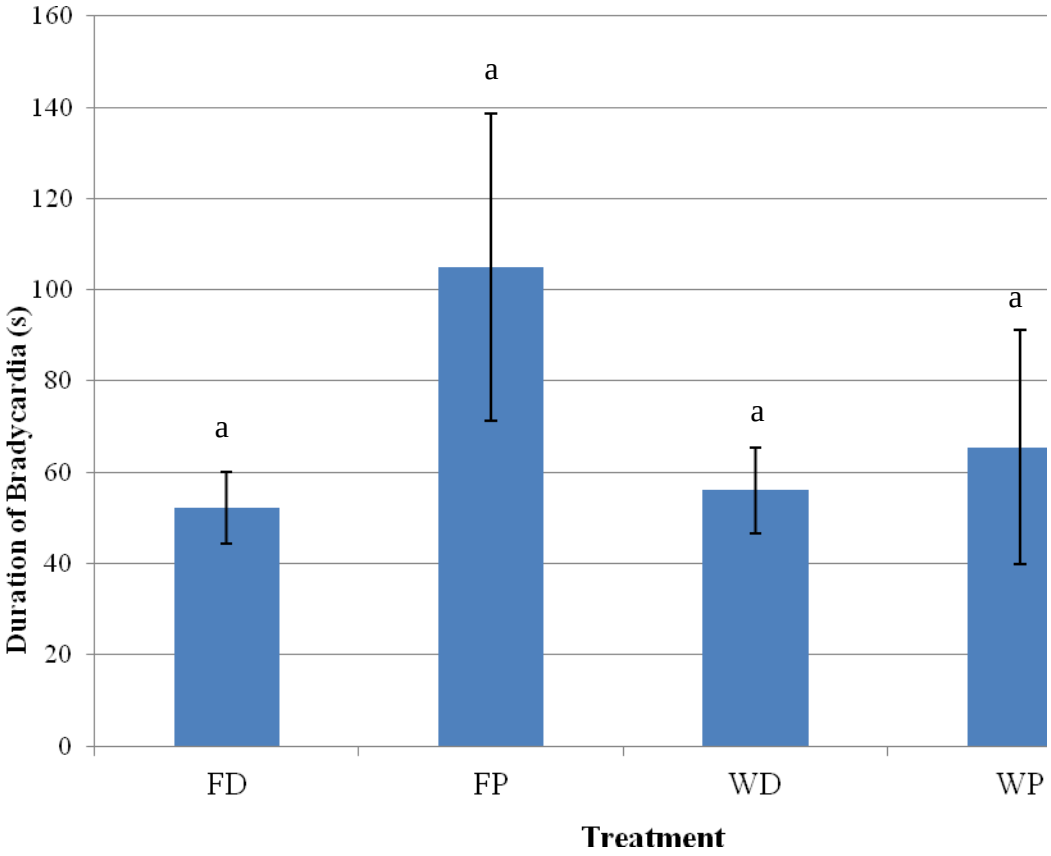

Figure 5 **Mean duration of bradycardia (seconds) observed in White Pekin ducks for each of the submersion treatments foam dunk (FD), foam pour (FP), water dunk (WD) and water pour (WP). Letters denote statistical significance between treatments ($\alpha = 0.05$).**

In addition to analyzing the effect of submersion of the ducks' bills in foam or water on heart rate, the duration of bradycardia caused by contact with foam or water was also analyzed. FP had no effect on duration of bradycardia across treatments ($p = 0.059$).

Table 3 **Difference in duration of bradycardia in seconds by four treatments: foam pour (FP), foam dunk (FD), water pour (WP), and water dunk (WD).**

Wilcoxon Scores (Rank Sum) for the Variable Duration (seconds) Classified by the Variable Treatment

Treatment	n	X	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
FD	17	52.2	455.5	552.5	65.7	26.8
WD	16	56.1	475.5	520.0	64.5	29.7
WP	16	65.5	494.5	520.0	64.5	30.9
FP	15	105.1	654.5	487.5	63.1	43.6

Kruskal-Wallis Test: Pr > Chi-Square = 0.059

The weight of the ducks (X = 2.8 kg, SEM = 0.05 kg) and the duration of bradycardia were negatively correlated ($r = -0.285$, $p = 0.023$). The data was also analyzed to determine if there was a correlation between the temperature of the water (X = 23.0 °C, SEM = 0.3 °C) and the duration of bradycardia. There was no correlation between water temperature and duration of bradycardia ($r = -0.087$, $p = 0.504$).

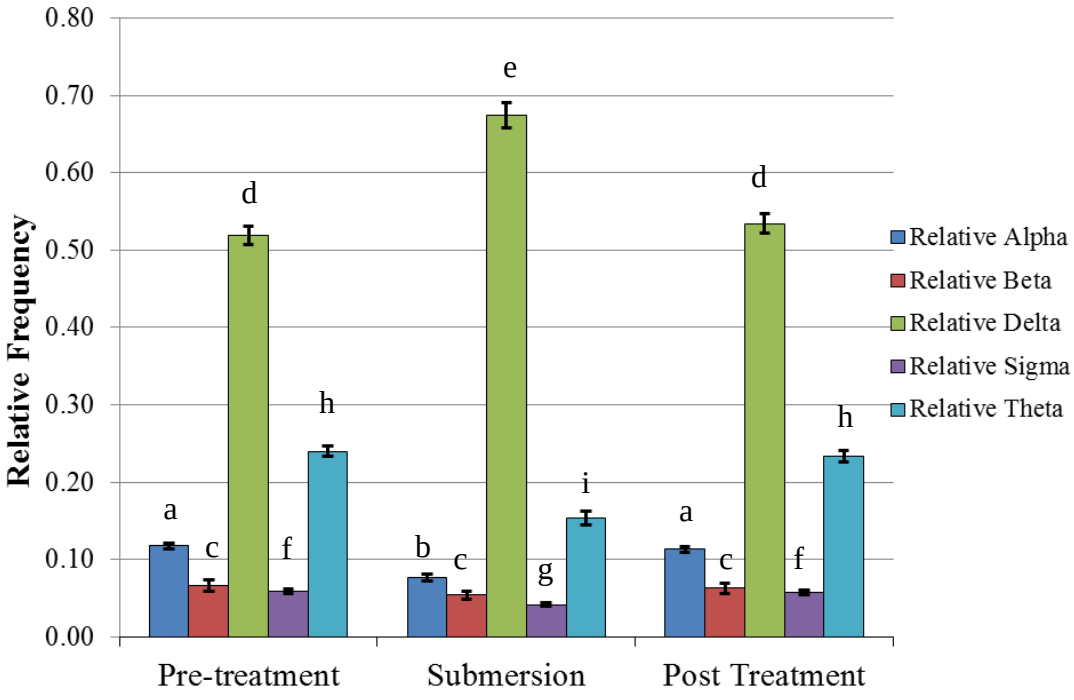

Figure 6 **Mean relative frequencies of the 4 treatments during the 420 s pretreatment, the 60 s submersion period and the 420 s post-treatment period. Data presented as mean ± standard error of the mean (X ± SEM). Letters denote statistical significance between periods (α = 0.05).**

The results of the frequency domain EEG analysis showed that there was a significant difference between the submersion period and the pre-treatment and post-

treatment periods for four of the five relative frequencies. Relative frequencies are the ratio of the individual frequency divided by the sum of the total frequencies, or the percent of an individual frequency out of the total signal. Relative frequencies normalize the results and account for differences in total signal power, allowing direct comparisons between treatments. Relative alpha was lower during the submersion period compared to pretreatment ($p < 0.001$) and post-treatment ($p < 0.001$) periods. Relative sigma was lower during the submersion period compared to pretreatment ($p < 0.001$) and post-treatment ($p < 0.001$) periods. Relative theta was lower during the submersion period compared to pretreatment ($p < 0.001$) and post-treatment ($p < 0.001$) periods. Relative delta, however, was higher during the submersion period compared to pretreatment ($p < 0.001$) and post-treatment ($p < 0.001$) periods. No differences occurred between pre-treatment and post-treatment periods for alpha ($p = 0.417$), delta ($p = 0.373$), sigma ($p = 0.751$), or theta ($p = 0.509$). There was no effect of submersion on relative beta frequency between the pretreatment and submersion ($p = 0.184$), pretreatment and post-treatment periods ($p = 0.749$), and post-treatment and submersion ($p = 0.310$).

Experiment 3: Depopulation with and without atropine

The third experiment resulted in 100% mortality for all 3 treatments. Analysis of the four physiological events showed that unconsciousness was the first event to occur in all three treatments. Unconsciousness occurred most quickly (X = 89 s) in the foam + atropine treatment, followed by the CO_2 treatment (X = 124 s), and finally the foam treatment (X = 134 s). Foam ($p = 0.004$) and gas ($p = 0.004$) took longer to cause unconsciousness as compared to foam + atropine, and no differences occurred ($p = 0.552$) in unconsciousness times between foam and CO_2 gas treatments as shown in Table 4.

Table 4 **Basic descriptive statistics, excluding outliers, for the variables measured pertaining to the three methods of depopulation: foam, foam + atropine injection, and CO_2 gas. Data presented as mean ± standard error of the mean (X ± SEM). Means within a row with different superscripts differ ($p < 0.05$)**

	Treatment		
Variable	Water-Based Foam	Foam + Atropine Injection	CO_2 Gas
Unconsciousness	134 ± 13^{a}	89 ± 7^{b}	124 ± 8^{a}
Motion Cessation	199 ± 16^{a}	156 ± 12^{b}	185 ± 15^{ab}
Brain Death	240 ± 12^{a}	171 ± 8^{b}	185 ± 8^{b}
ATCA	265 ± 19^{a}	188 ± 10^{b}	253 ± 12^{a}
Duration of Bradycardia	156 ± 14^{a}	N/A	78 ± 11^{b}
Pre-treatment Heart Rate (bpm)	258 ± 17^{a}	272 ± 12^{a}	210 ± 13^{b}
Onset of Bradycardia to Onset of Unconsciousness	114 ± 15^{a}	N/A	84 ± 14^{a}
Age (days)	85 ± 2^{a}	79 ± 2^{ab}	74 ± 3^{b}

The time elapsed between the start of treatment and the occurrence of motion cessation occurred first (X = 156 s) for the treatment foam + atropine. Motion cessation occurred at X = 185 s for CO_2 gas and at X = 199 s for foam. The elapsed time to motion cessation was longer for foam compared to foam + atropine ($p = 0.040$). No differences occurred between CO_2 gas and foam + atropine ($p = 0.129$) or foam and CO_2 gas ($p = 0.562$).

The event brain death again occurred within the shortest period of time (X = 171 s) for the treatment foam + atropine, followed by CO_2 gas (X = 185 s) and then foam (X = 240 s). Brain death differs from unconsciousness and motion cessation in that foam caused a longer elapsed time to brain death as compared to foam + atropine ($p < 0.001$) and CO_2 gas ($p < 0.001$). No differences occurred between CO_2 gas and foam + atropine ($p = 0.235$).

The occurrence of ATCA, the cessation of rhythmic electrical cardiac activity that invariably results in death, was the fastest for foam + atropine (X = 188 s), making foam + atropine faster than CO_2 gas (X = 253 s, $p < 0.001$) and foam ($\mu = 265$ s, $p < 0.001$). No differences occurred in elapsed time to ATCA between foam and CO_2 gas ($p = 0.598$).

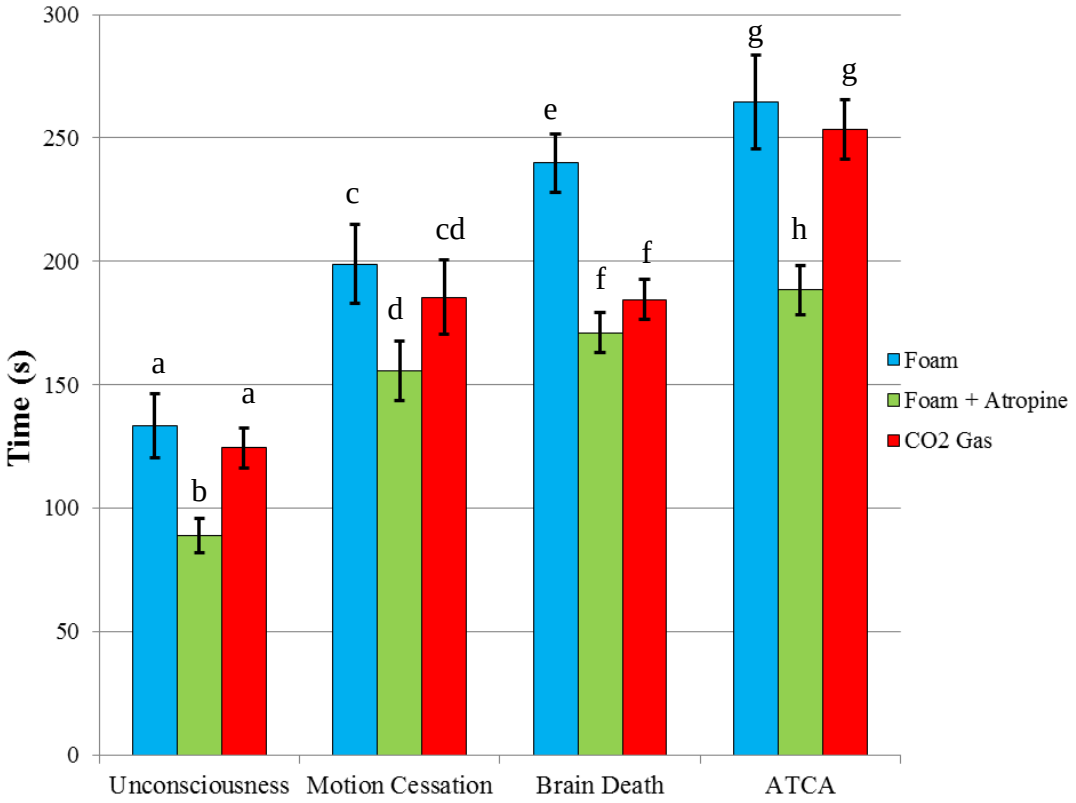

Figure 7 **Mean time in seconds to the physiological points unconsciousness, motion cessation, brain death, and ATCA for White Pekin ducks after depopulation with foam, foam + atropine, or CO_2 gas. Data presented as mean ± standard error of the mean (X ± SEM). Letters denote statistical significance between treatments ($p < 0.05$).**

The duration of bradycardia was determined for each treatment as described in Materials and Methods. The mean duration of bradycardia was the longest for foam (μ = 156 s), followed by CO_2 gas (μ = 78 s) ($p < 0.001$). Bradycardia did not occur in ducks treated with foam + atropine injection.

The pre-treatment heart rate (HR) was recorded for each duck 60 s prior to depopulation and compared between treatments. The foam + atropine treatment had the highest pre-treatment HR (X = 272 bpm), followed by foam (X = 258 bpm), and finally CO_2 gas (X = 210 bpm). The CO_2 gas treatment had a lower pre-treatment HR than both foam + atropine ($p = 0.002$) and foam ($p = 0.035$). No differences occurred between the pre-treatment HR of the foam and foam + atropine treatment ($p = 0.503$).

The elapsed time between the onset of bradycardia and the onset of unconsciousness was also analyzed as described in Materials and Methods. Bradycardia did not occur in the treatment foam + atropine, and was therefore not included in this analysis. No differences occurred between the foam and CO_2 gas treatments ($p = 0.119$).

Age in days was the final variable analyzed between the three treatments. The mean age for the foam treatment was the oldest (X = 84.7 days), followed by foam + atropine (X = 78.5 days), and finally CO_2 gas at the youngest (X = 73.6 days). The

foam treatment was older than the CO_2 treatment ($p = 0.022$). There was no difference in age between the CO_2 gas and foam + atropine treatments ($p = 0.219$) or foam and foam + atropine ($p = 0.137$).

The results of Experiment 3 showed that there was a positive correlation between time to unconsciousness and time to motion cessation ($r = 0.615$, $p < 0.001$), time to unconsciousness and time to brain death ($r = 0.792$, $p < 0.001$), and time to unconsciousness and time to ATCA ($r = 0.682$, $p < 0.001$). There was a positive correlation between time to motion cessation and time to brain death ($r = 0.650$, $p < 0.001$) and time to motion cessation and time to ATCA ($r = 0.701$, $p < 0.001$). There was a positive correlation between time to brain death and time to ATCA ($r = 0.653$, $p < 0.001$). There was also a positive correlation between duration of bradycardia and time to unconsciousness ($r = 0.657$, $p < 0.001$), duration of bradycardia and time to motion cessation ($r = 0.562$, $p < 0.001$), duration of bradycardia and time to brain death ($r = 0.611$, $p < 0.001$), and duration of bradycardia and time to ATCA ($r = 0.590$, $p < 0.001$). There was a negative correlation between duration of bradycardia and pre-treatment HR ($r = -0.383$, $p = 0.012$). The time between onset of bradycardia and onset of unconsciousness was positively correlated with time to unconsciousness ($r = 0.926$, $p < 0.001$), time to motion cessation ($r = 0.617$, $p < 0.002$), time to brain death ($r = 0.814$, $p < 0.001$), and time to ATCA ($r = 0.562$, $p = 0.004$). Age was negatively

correlated with time to unconsciousness ($r = -0.374$, $p = 0.021$), time to brain death ($r = -0.354$, $p = 0.022$), and positively correlated with pre-treatment HR ($r = 0.360$, $p = 0.027$). Pre-treatment HR was negatively correlated with time to unconsciousness ($r = -0.348$, $p = 0.032$) and time to ATCA ($r = -0.488$, $p = 0.001$).

Chapter 4

DISCUSSION

The objective of Experiment 1: Depopulation was to determine if there was a significant difference in time to unconsciousness, motion cessation, brain death, and ATCA between water-based foam and CO_2 gas for White Pekin ducks. In Experiment 1, ducks were depopulated using either CO_2 gas or water-based foam, and the time it took to reach unconsciousness, motion cessation, brain death, and the appearance of altered terminal cardiac activity (ATCA), defined here as the cessation of rhythmic electrical cardiac activity that invariably results in death, was measured. It was found that CO_2 was significantly faster than foam for all physiological points measured, including unconsciousness and brain death. A similar experiment was performed by Benson et al. (2009) with both White Pekin ducks and call ducks, but water-based foam was not directly compared to CO_2, and EEG was not used in these experiments to determine the onset of unconsciousness and brain death (Benson et al., 2009). When comparing the results from Experiment 1 to the experiment performed by Benson et al. (2009), the elapsed times to ECG suppression in foam for Pekin ducks of unknown age (μ = 368.7 s) were longer than the elapsed time to ATCA in foam for

Pekin ducks ranging from 5-9 weeks of age (μ = 300 s). The variations seen could be due to the age or weight of the ducks, as well as the method by which the foam was applied to the duck, slight variations in foam consistency, and the differences in the way that ATCA and ECG silence were assessed. Overall, however, the times appear to be consistent when comparing foam only. The results of Experiment 1, which included CO_2 gas, were inconsistent with previous studies, that showed that water based foam was typically similar to or slightly faster than CO_2 gas for depopulation of chickens and turkeys (Alphin et al., 2010; Benson et al., 2007; Rankin, 2010). While Figures 2 and 3 are representative examples, 95% of ducks depopulated in foam in Experiment 1 showed cardiac patterns similar to those seen in Figure 2 within 10-30 s of foam application, while only 50% of ducks depopulated with CO_2 in Experiment 1 showed this pattern. Since there was a recovery in heart rate before ATCA occurred, it seems likely that physiological mechanisms, such as the diving reflex, may be contributing to the differences seen. It should also be noted that similar patterns of pronounced bradycardia were not seen in other species.

The objective of Experiment 2: Submersion was to determine if submersion of the bill in foam causes a diving reflex including bradycardia or other cardiac patterns similar to submersion in water, as well as provide a baseline to measure future cardiac adjustments as compared to diving bradycardia produced by

submersion of the bill in water. In addition, this experiment sought to determine if static contact of the bill in foam was sufficient to trigger a diving reflex, causing cessation of respiration and bradycardia.

An experiment by Hudson and Jones (1986) showed that there is a direct correlation between body mass in the Pekin duck and endurance during a restrained forced dive with a similar set up to this experiment. The equation used to describe this relationship is:

$$t_d = 6.6\ M_b^{0.64}$$

Where t_d is dive time in minutes and M_b is body mass in kilograms. Based on this equation, $t = 6.6(2\ kg)^{0.64} = 5.2$ minutes; a forced dive time of 60 s should not be beyond the capabilities of 8 week old ducks, since according to a growth chart 56 day old White Pekin ducks should weigh approximately 2.0 to 2.5 kilograms (Gille & Salomon, 2000). Experiment 2 used White Pekin ducks that weighed between 2.0 and 3.2 kg during the course of the experiment.

During Experiment 2, all of the ducks tested using the treatments foam pour (FP), foam dunk (FD), water pour (WP), and water dunk (WD) developed bradycardia during the 60 s treatment period, which occurred from 420-480 s. The

results showed that there was no significant difference between any of the treatments during the final 30 s of the treatment period (450-480 s), indicating that all treatments caused a similar level of bradycardia.

The graph in Figure 4 shows that FD and WD caused lower heart rates during the first 30 s of the treatment period (420-450 s) as opposed to FP and WP. These results suggest that the action of dunking the bill in the water or foam lead to a faster induction of bradycardia as compared to pouring water or foam over the bill. FP resulted in a significantly lower heart rate during the first 30 s of the post-treatment period (480-510 s) compared to FD and WD. WP resulted in a significantly lower heart rate during this period (480-510 s) compared to FD. Additionally, the significant difference in heart rate during the final periods of post-treatment recovery (840-870 s and 870-900 s) between FP and WD are most likely due to the water-based foam sticking to the bill, leading to a prolonged response and a delay in the appearance of the higher heart rate seen during the recovery periods of the other treatments.

Since there was no significant difference in heart rate between FP and WP or FD and WD, it can be concluded that the mechanism by which the foam or water was applied to the bill made a greater difference in heart rate drop and recovery as opposed to the type of liquid. These results are important because they show that heart rate can be affected when pouring foam directly over the ducks' heads, as in individual

laboratory depopulation such as Experiments 1 and 3 and in contrast to foam rising from the ground and building above the birds' heads. Experiments 1 and 3 used a Spumifer nozzle suitable for depopulation in the field that had a rate of 1136 L/min, which is much greater than the flow rate of 13.3 L/min achieved during Experiment 2. The movement of the foam across the bill of the duck during laboratory depopulations such as Experiments 1 and 3 occurs within a few seconds due to the higher flow rate of the Spumifer nozzle, which can be seen in the motion artifacts of ECG tracings obtained during both depopulation experiments. In the field, however, foam is typically directed onto the ground or the sidewall of a large pen or building and allowed to build up to a level above the birds' heads (Benson et al., 2009; Simunich, 2009), which may mimic the low flow rate of Experiment 2. Based on the graph shown in Figure 4, foam depopulation in which foam is applied directly to the ducks' bodies or bills would most likely result in a slower diving response, whereas a situation where the foam was allowed to slowly engulf the ducks would most likely result in a fast diving response as soon as the bill was dipped into the foam.

It is important to note that all ducks that were tested survived the submersion in both water and foam. Experiment 2 has shown that submersion of White Pekin ducks in both foam and water leads to an initial apnea that lasted 60 s, the time at which the bill was removed from the foam and the duck recovered.

Submersion in foam, therefore, initially causes voluntary apnea, as opposed to apnea caused by the foam occluding the trachea, and has similar cardiac effects to submersion in water. According to Suzuki (1996), terminal submersion of mammals (dogs and rabbits) in water occurs in four stages: surprise-respiration and initial apnea, dyspnea, apnea, and finally terminal respiration (Suzuki, 1996). Based on the results of the current three experiments, ducks may experience the same surprise-respiration, which causes the foam (or water) to contact and stimulate the glottis, which leads to the initial apnea. It can therefore be concluded that foam depopulation, because it involves full submersion in water-based foam, causes a diving reflex in White Pekin ducks. Whether this initial apnea, followed by unconsciousness and death, is stressful or not remains to be determined.

The EEG results from Experiment 2 show that there is a significant increase in the relative delta frequency during submersion, along with a significant decrease in the relative alpha, sigma, and theta frequencies. A rise in delta along with a decrease in alpha has been previously shown to correlate with unconsciousness (Benson et al., 2012). The ducks in Experiment 2, however, remained consciousness throughout the experiment. The frequency changes in Experiment 2 may be due to the effects of hypoxia or possibly be due to a stress response via an unknown mechanism. Further testing is still required to replicate the results of Experiment 2 with other

potentially stressful stimuli and correlate the changes in relative EEG frequencies with changes in corticosterone levels. While testing has shown that both foam and CO_2 gas depopulation causes similar levels of stress in broilers (Benson et al., 2007), the three experiments performed in the current study did not measure corticosterone levels, and further work is required to determine the level of stress caused by foam and CO_2 gas depopulation in White Pekin ducks and other waterfowl.

The objective of Experiment 3 was to determine the effects of submersion bradycardia on the time to four physiological events that occur during depopulation by adding the third treatment, foam + atropine. Experiment 3 was performed in addition to Experiment 1 because the results from Experiment 1 showed that CO_2 gas was significantly faster than foam, which was different from the results found in gallinaceous birds such as chickens (Benson et al., 2007) and turkeys (Rankin, 2010). The ECG tracings from Experiment 1 also indicated that the ducks treated with foam developed bradycardia before losing consciousness, which was not observed in other species. Experiment 3 also required the addition of a third treatment, foam + atropine injection, to determine the effects of abolishing bradycardia in ducks depopulated with foam. This third treatment needed to be directly compared to the other two treatments, foam and gas, and weight and age needed to be better recorded in the new experiment to evaluate any effects of these two variables. In addition to measuring time to death,

the onset and duration of bradycardia, pre-treatment heart rate, age, and weight of the ducks was analyzed. The elapsed time between the onset of bradycardia and the onset of unconsciousness was also measured.

Atropine sulfate is used during cardiac emergencies at 0.5 mg/kg intramuscularly, intravenously, or intraosseously to increase heart rate during bradyarrhythmias with fast effects (Manual of Avian Medicine, 2000). Butler and Jones (1971) and Bryan and Jones (1980) used atropine sulfate at a dosage of 2.5 mg/kg to prevent bradycardia during diving in Pekin ducks with no adverse side effects reported. In a study by Lin and Horvath (1972), atropine sulfate (1 mg/kg) was injected intraperitoneally into rats to block autonomic nervous activity; peak heart rates were recorded after 20 minutes (Lin & Horvath, 1972). For most species of birds, the effects of atropine sulfate are rapid and decline after 30 minutes (Manual of Avian Medicine, 2000). The protocol used in this experiment for treating the ducks with atropine was based on these previous studies.

Ducks injected IM with 0.5 mg/kg of atropine sulfate (0.54 mg/mL) 900 s prior to the application of foam showed no difference in HR (bpm) during the 60 s pre-depopulation baseline as compared to untreated ducks in the foam treatment, however, it was significantly higher than the CO_2 gas treatment. This was most likely not due to the effects of the atropine because the CO_2 gas treatment had a significantly lower pre-

treatment HR than both the foam and the foam + atropine treatments. These differences may have been due to a stress caused by ambient researcher activity, since in-lab foam depopulation required loud verbal communication between the person in the lab working the foam nozzle and the person outside working the foam generator. Ducks in the foam + atropine treatment also had to be handled more extensively because although they were caught and immobilized similar to ducks in other treatments, they were given an injection.

Atropine provided complete blockage of bradycardia, as none of the ducks in the atropine + foam treatment developed bradycardia after the application of foam. The ducks in the foam treatment, however, developed bradycardia in every trial. Ducks in the CO_2 gas treatment developed bradycardia in 20 out of 23 trials (87%). This result was unexpected; during Experiment 1 only 9 out of 18 (50%) ducks depopulated using CO_2 gas showed bradycardia on the ECG. The results from Experiment 1 were more consistent with Experiment 3 for foam in that 18 out of 19 (95%) ducks developed bradycardia in response to foam depopulation, while in Experiment 3, 19 out of 19 ducks (100%) developed bradycardia in response to foam.

There have been several experiments performed in the past that have examined the effects of hypercapnia and hypoxia on ducks, which offer some explanation of the results obtained in the current experiments. One such experiment,

performed in 1913, showed that ducks responded to hypercapnia with decreased respiration and apnea (Orr & Watson, 1913). The levels of CO_2 used in these experiments were between 5 and 20% and the CO_2 was administered both through an endotracheal tube as well as in a sealed box into which the duck was placed. It was later found that the unexpected result of apnea in response to inhalation of CO_2 was caused by the irritant properties of the gas itself, as opposed to its effects on chemoreceptors (Dooley & Koppanyi, 1929). When CO_2 was directly injected into the ducks' bloodstreams, effectively bypassing the trachea, nares, and glottis, respiration increased, as expected, rather than decreased in response (Dooley & Koppanyi, 1929). These results were supported by a later study by Jones and Purves (1970) when CO_2 gas was administered through an endotracheal tube, resulting in an increase in expired volume as well as a decrease in respiratory frequency and heart rate (Jones & Purves, 1970).

The results of these past studies provide a logical explanation for the results observed in the current study. The ducks in the current study were placed in a chamber that was filled with a high concentration of CO_2 gas, which could have stimulated the nasal passages and glottis, potentially triggering apnea and therefore bradycardia similar in appearance on the ECG to what was observed in the foam treatment.

The reason for a higher percentage of ducks showing bradycardia in response to CO_2 gas depopulation in Experiment 3 as compared to Experiment 1could also be due to the age difference. The age range for the ducks used in Experiment 1 was 5 to 9 weeks of age, while the ducks used in Experiment 3 were between 8 and 14 weeks of age. The results for Experiment 3 show that while age was not directly correlated with duration of bradycardia, it was correlated with the pre-treatment HR. Since pre-treatment HR was correlated with duration of bradycardia, it cannot be completely ruled out that age has no effect on bradycardia. Age was also correlated with time to unconsciousness and time to brain death, as was duration of bradycardia.

Based on the results of both Experiment 1 and Experiment 3, it appears that the age of the ducks potentially had some influence on time to unconsciousness and brain death. The duration of bradycardia, which was significantly longer in ducks depopulated with foam as opposed to CO_2 gas (Table 1, Figure 1, Table 4, Figure 7), also lengthened the time to the four physiological points for foam ducks in both Experiment 1 and Experiment 3 as compared to the foam + atropine treatment. The mean time to unconsciousness for the 5-9 week old foam ducks (142 s) and the 8-14 week old foam ducks (134 s) was greater than the mean time to unconsciousness for 8-14 week old foam + atropine ducks (89 s). The same trend was seen for time to brain death as well (young, foam = 283 s; old, foam = 240 s; old; foam + atropine = 171 s).

Within the same age group (8-14 weeks old), foam + atropine was significantly faster than foam (Table 4, Figure 7). The older group of ducks, however, also showed a trend in which they appeared to be more resistant to CO_2 gas but slightly more susceptible to water-based foam than the younger group of ducks. Older ducks (Experiment 3) depopulated with foam reached unconsciousness (old, foam = 134 s; young, foam = 142 s), motion cessation (old, foam = 199 s; young, foam = 243 s), brain death (old, foam = 240 s; young, foam = 283 s), and ATCA (old, foam = 265 s; young, foam = 300 s) more quickly than younger ducks (Experiment 1) depopulated with foam. It is possible that these results were caused by the small sample size of the treatment groups. Additional testing would need to be performed to determine if this trend is due to an age effect, or if it is due to variability within the sample sizes.

When deciding on a method to use for mass emergency depopulation of floor-reared poultry, there are many factors to take into consideration. The results of these experiments show that it is important to consider the species of bird being depopulated as well as the age of the birds. Also of great importance is the type of housing and climate in which the birds are kept. Deciding whether foam or CO_2 gas will be more effective for depopulation depends highly on each situation, as both methods have advantages and disadvantages. It is important to remember that the results of these three experiments apply only to individual ducks being depopulated in

a lab. The lack of scientific studies performed specifically with waterfowl in whole house depopulation situations may make the decision more complicated, but the following studies of mass depopulation of gallinaceous poultry may aid in making a decision based on housing type.

Depopulation of floor-reared poultry using water-based foam offers several advantages over CO_2 gas. When antibodies to H5N2 (West Virginia) and H5N1 (Virginia) was detected during routine pre-slaughter testing in 2007, water-based foam was used to depopulate 25,560 turkeys and 54,000 turkeys respectively (Flory and Peer, 2010). The use of foam during these two outbreaks was found to be fast and effective, and the additional water used to create the foam was ideal for in-house composting of the carcasses. For the West Virginia depopulation, contaminated carcasses and litter did not leave the buildings, few people needed to enter the buildings, and the foam equipment was able to be disinfected. These qualities enhanced the biosecurity of the operation, which is essential for containing an outbreak (Alphin et al., 2010; Flory & Peer, 2010). Even moving the birds just outside the house can compromise biosecurity (Alphin et al., 2010). Implementing foam depopulation is also fast and requires fewer personnel, especially compared to partial house and containerized gassing. When dealing with structurally damaged houses or the open air houses found in warm climates, this aspect becomes a significant

advantage as compared to whole house CO_2 gassing. During an outbreak of LPAI H5N8 on a game bird farm in Idaho, water-based foam was used to depopulate ducks, pheasants, and chukars that were living in open air structures and pens that were not able to be sealed (Simunich, 2009). The use of foam during this incident allowed the personnel to rapidly depopulate the infected birds with minimal handling.

While foam has many advantages, especially in structurally damaged or open air houses, it also has some disadvantages. Water is essential for foam depopulation and aids in composting, however, it is important to identify sources of water that are of good quality and sufficient quantity to perform the depopulation (Flory & Peer, 2010). A worst case scenario for the industry is represented by a 122 m long house containing turkeys at market weight (18 kg), which would require approximately 30,000 L of water to depopulate; it is therefore important to identify these water sources prior to an outbreak (Flory & Peer, 2010). In addition to the water requirement, the foam generating equipment itself should be tested on a regular basis to ensure it is in proper working order should an emergency arise (Flory & Peer, 2010). Despite the significantly lower personnel requirements that the foam equipment demands, the personnel must be properly trained in its use, and these personnel still need to enter the house in personal protective equipment to administer

the foam and move the birds out of the path of the cart (Dawson et al., 2006, Flory & Peer, 2010).

Most of the disadvantages associated with foam depopulation are logistical; however, some concern has been raised about the welfare of birds depopulated with water-based foam. Raj et al. (2008) states that occlusion of the trachea- the mechanism by which water-based foam leads to death- is equivalent to death by drowning or suffocation, methods not recognized as humane under European legislation (Raj et al., 2008). Studies of foam depopulation have stated that inhalation of the foam does not cause drowning because dye was found in the trachea but not in the lungs of broilers depopulated using dyed water-based foam (Benson et al., 2007). A type of drowning called "dry" drowning, however, can occur without liquid entering the lungs (Ludders et al., 1999). Dry drowning does appear to be caused by a different mechanism than death by inhaling foam, since dry drowning occurs when a liquid is inhaled and causes laryngospasm which seals the trachea and prevents the liquid from entering the lungs (Ludders et al., 1999; Suzuki, 1996; Yagil et al., 1983). As stated above, submerging broilers in foam lead to foam entering and occluding the trachea (Benson et al., 2007); therefore the mechanism of death is more similar to suffocation as it leads to death by mechanical hypoxia.

Whole house, partial house, and containerized gassing using CO_2 are common methods of depopulation that also have some advantages and disadvantages. Whole house CO_2 gassing has benefits such as the following: it is highly effective, can depopulate an entire house of poultry at one time, reduces the number of personnel required to enter the house and can eliminate the need to handle birds that are infected with virus as long as it can be completely sealed from the outside (Gerritzen et al. 2006a, McKeegan et al. 2011; Ryan et al. 2006). An exercise performed in Kilmeedy County, Limerick, Ireland, used a novel lance assembly to inject liquid CO_2 from a tanker into a house of 12,000 broiler breeders, and the house was sealed from the outside (Ryan et al. 2006). Gerritzen et al. (2006a) found that it was possible to increase the CO_2 concentration in large houses to the recommended 40% in breathing air for chickens in approximately 30 min, which was highly effective for killing (Gerritzen et al. 2006a). At the time, it was unclear if this concentration of CO_2 would be effective for killing ducks and turkeys, but a later study showed that the progressive hypercapnia combined with severe hypoxia and acidosis caused by gradual introduction of CO_2 gas was able to cause unconsciousness and kill both ducks and turkeys at similar concentrations (Gerritzen et al. 2006b). As a result of this study, Gerritzen et al. (2006b) adjusted the recommended concentration of CO_2 gas to 45% in breathing air for 30 min for all farmed poultry (Gerritzen et al. 2006b). The results of

the study by Gerritzen et al (2006b) support the findings of the current three experiments.

One of the main disadvantages associated with CO_2 gas is the reaction of birds and other animals to the gas itself. Many studies and emergency depopulation situations that have used CO_2 gas, regardless of method of introduction, have found that the birds displayed reactions to CO_2 such as headshaking and gasping (Gerritzen et al., 2004; McKeegan, 2004; McKeegan et al., 2006; McKeegan et al., 2011; Raj, 1996; Raj et al., 2006). These reactions are typically considered aversive and unpleasant, both for the poultry and the human observers. It is known that inhalation of CO_2, in birds as well as mammals, stimulates central and peripheral chemoreceptors, which causes the respiratory response seen as gasping (McKeegan et al., 2011). A previous study by McKeegan et al. (2006) tested the reactions of chickens to stimulation with the inert gasses N_2 and Ar, as well as CO_2. Gasping was not observed in birds exposed to the inert gasses, however, headshaking was observed in a few birds, indicating that headshaking may be a behavioral response to a novel or alerting stimulus as opposed to respiratory distress (McKeegan et al., 2006). When respiration was disturbed as a result of CO_2 exposure, birds continued to stay in the vicinity of a feed dish and even kept feeding until concentrations reached 40%, which suggested that the gasping reflex may not be aversive (McKeegan et al., 2006).

Gasping was seen at all concentrations of CO_2, even when mixed with the inert gasses, and so it was determined that respiratory disruption was not likely the cause of the withdrawal response, but rather trigeminal sensitivity at concentrations above 40% (McKeegan, 2004; McKeegan et al., 2006). Even so, to avoid potential discomfort, Gerritzen et al. (2004) recommends a gradual increase of CO_2 to 17% in the breathing air to induce loss of posture (Gerritzen et al., 2004). A study performed by McKeegan et al. (2011) sought to more accurately gauge the physiological effects of whole house CO_2 gassing by depopulating 28,000 spent laying hens using liquid CO_2 (McKeegan et al., 2011). While it was found that the hens experienced deep breathing as the concentration of CO_2 rose, the 20% concentration of CO_2 achieved during consciousness did not reach the 40% needed to stimulate nasal and oral nociceptors, which would induce pain (McKeegan, 2004; McKeegan et al., 2011).

Another welfare concern related to whole house CO_2 gassing is the potential for birds to experience hypothermia or even freeze when exposed to the vapor given off by compressed or liquid CO_2. Additionally, to achieve the proper gas concentrations, the ventilation system must be switched off and the vents sealed, which may lead to uncomfortable increases in both temperature and ammonia levels in the house (Raj et al., 2006). During the depopulation performed by Ryan et al. (2006), the vents were left open and the tops unsealed until immediately before gassing, and

after gassing concluded no frozen birds were found. Hypothermia was not directly assessed as cloacal temperatures were taken post-mortem, however, it was calculated from sensors that were placed throughout the house that birds were exposed to temperatures as low as -5.8 °C while conscious (Ryan et al., 2006). The layer depopulation performed by McKeegan et al. (2011) also monitored bird temperature to determine whether birds experienced hypothermia during whole house CO_2 gassing. While the results from that specific depopulation may differ when performed in a non-caged housing system, the latest time to loss of consciousness occurred significantly earlier than any temperature readings less than 0°C and cloacal temperatures indicated that the birds did not die of hypothermia (McKeegan et al., 2011).

Partial house gassing offers a different advantage as compared to whole house gassing. Partial house gassing, which requires the construction of a polyethylene tent over the birds, does make it possible to effectively concentrate the CO_2 gas in an open air or structurally damaged house that cannot be sealed to levels capable of causing unconsciousness and death. The creation of such structures, however, requires an estimated 40 people to depopulate a farm housing 75,000 to 100,000 broilers in 1 day, and these personnel would all have to enter the house, corral and handle the birds, and then disinfect and dispose of the polyethylene sheeting (Alphin et al., 2010; Benson et al., 2007; Ritter, 2004). Partial house gassing,

therefore, offers a low level of biosecurity with a high level of labor (Alphin et al., 2010).

Containerized gassing offers the advantage of being able to better control the rate of gas increase for a smoother, faster induction, and because of this is more compatible with other inert gasses such as nitrogen and argon (Gerritzen et al., 2006a; Raj, 2008). While containerized systems allow this atmospheric control, they also require birds to be caught by hand using a catching crew (Gerritzen et al., 2006a; Raj, 2008). The birds must then be carried out of the house, exposing the personnel to the virus as well as potentially releasing the virus from within the confines of the house, which compromises biosecurity (Alphin et al., 2010; Gerritzen et al., 2006a). Not only does containerized gassing compromise biosecurity and human health, it is also associated with welfare concerns for the birds, since catching and handling can cause fear and pain due to bruising and broken bones (Boissy, 1995; Knowles & Broom, 1990).

When considering the safety, emotional impact, and speed of the emergency depopulation from the human perspective, foam clearly has many advantages over CO_2 gassing as discussed above. The safety of the personnel involved should always be considered first, such as in the event of a structural failure when a house may be unsafe to enter. The welfare of the birds and public perception,

however, are also important and when a situation is not immediately dangerous to the personnel involved, should be regarded with great care as well. It is for this reason that determining the time to unconsciousness has become so important. Unconsciousness is an important physiological state because the faster the bird reaches unconsciousness, the faster they are insensible to outside stimuli that may cause pain or stress. From a welfare standpoint, the results of these three experiments show that triggering the diving response when depopulating ducks should be avoided if possible. As Experiment 3 showed, ducks that did not develop bradycardia reached unconsciousness significantly faster than ducks that did. That experiment also showed, however, that both CO_2 gas and water-based foam can cause bradycardia and significantly prolong time to unconsciousness. When depopulating ducks using CO_2 gas, this may be able to be avoided by gradually increasing the concentration of gas to no more than 20% of the breathing air until the birds reach unconsciousness or loss of posture so that the nasal and oral nociceptors are not stimulated (McKeegan, 2004; McKeegan et al., 2011). Further testing, particularly in a field situation, would be needed to confirm this method. It should also be noted that in Experiment 1, significant differences between the foam and CO_2 treatment for the time between onset of bradycardia and onset to unconsciousness were found, indicating that the younger ducks (5-9 weeks) were both conscious of the foam treatment and the

physiological response it caused for a longer period of time than the CO_2 treatment. For Experiment 3, however, no significant differences between the foam and CO_2 treatment for the time between onset of bradycardia and onset to unconsciousness occurred, indicating that the older ducks (8-14 weeks) were not aware of the effects of one treatment for much longer than the other. These differences may not hold true under field conditions, however, since it would almost certainly take longer for CO_2 gas concentrations to rise to levels high enough to cause unconsciousness during whole house depopulation.

Chapter 5

CONCLUSIONS

The depopulation of production birds is an effective and important method of preventing the spread of highly infectious diseases. Depopulating poultry that are infected or potentially infected in a quick and timely manner prevents an excessive loss of life and economic disaster. While human safety must first be taken into consideration, the welfare of the birds is also important to ensure that the method of depopulation leads to unconsciousness and brain death in the least amount of time possible. The two common methods of depopulation, CO_2 gassing and water-based foam, both have advantages and disadvantages in the field and therefore each situation needs to be fully evaluated before choosing a method. Experiment 1 evaluated the difference in time to unconsciousness, motion cessation, brain death, and ATCA between foam and CO_2 gas for White Pekin ducks aged 5-9 weeks. Experiment 1 did not support the hypothesis that no differences would occur between water-based foam and CO2 gas treatments for times to unconsciousness, motion cessation, brain death, and ATCA. Experiment 2 evaluated the effects of non-terminal submersion in both flowing and standing water and water-based foam on heart rate and duration of

bradycardia. Experiment 2 supported the hypothesis that water-based foam triggers the diving reflex, resulting in bradycardia. Experiment 3 evaluated the effect of abolishing bradycardia on difference in time to unconsciousness, motion cessation, brain death, and ATCA between foam, CO_2 gas and foam + atropine injection. Experiment 3 did not support the hypothesis that blocking bradycardia would result in no difference in time to unconsciousness and death between the foam treatment and CO_2 gas treatment, however, it did support the hypothesis that bradycardia has a salvific effect during depopulation. These three experiments show that water-based foam can be an effective and efficient means of depopulation, even for White Pekin ducks.. The drawback of using water-based foam to depopulate White Pekin ducks, however, is that these three experiments show that water-based foam does cause an initial period of apnea and bradycardia, which prolongs the time to unconsciousness and death. Since CO_2 gassing can also cause apnea and bradycardia when the concentration rises rapidly, this method does not always lead to a shorter time to unconsciousness than foam, and in Experiment 3, there was no difference in times to unconsciousness and death between CO_2 and water-based foam in White Pekin ducks in experimental situations with small sample sizes. It can therefore be concluded that bradycardia may occur regardless of method and extra exposure time should be allowed to compensate for this response.

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APPENDIX

UNIVERSITY OF DELAWARE

COLLEGE OF AGRICULTURE AND NATURAL RESOURCES

AGRICULTURAL ANIMAL CARE AND USE COMMITTEE

Application for Use of Agricultural Animals

In Teaching or Research

AACUC Protocol Number: (33) 05-02-11R

TITLE OF PROJECT: Evaluation of the diving reflex in response to water-based foam vs. carbon dioxide depopulation in White Pekin ducks

INSTRUCTOR/PRINCIPAL INVESTIGATOR

Eric R Benson — 6/7/2011

Printed Name **Signature** **Date**

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(This section for Committee use only)

Application Approved (date) 6/20/2011

Application Rejected (date) _____

Reason for Rejection _____

_____ _____

Signature, Animal Care and Use Committee **Date**