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ASPECTS OF WHITE-TAILED DEER ECOLOGY AND MANAGEMENT: EFFECTS OF IMIDACLOPRID AND AN EVALUATION OF MANAGEMENT AT GREAT SWAMP NATIONAL WILDLIFE REFUGE

 $\mathbf{B}\mathbf{Y}$

ELISE HUGHES BERHEIM

A thesis submitted in partial fulfillment of the requirements for the

Master of Science

Major in Wildlife and Fisheries Sciences

Specialization in Wildlife Sciences

South Dakota State University

2018

ASPECTS OF WHITE-TAILED DEER ECOLOGY AND MANAGEMENT: EFFECTS OF IMIDACLOPRID AND AN EVALUATION OF MANAGEMENT AT GREAT SWAMP NATIONAL WILDLIFE REFUGE

ELISE HUGHES BERHEIM

This thesis is approved as a creditable and independent investigation by a candidate Master of Science in Wildlife and Fisheries Sciences degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Jonathan Jenks, PK-D. Thesis Advisor

Date

Michele Dudash, Ph.D. Head, Department of Natural Resource Management Date

Dear, Graduate School

Date

Dedicated to the Deer in this Study

ACKNOWLEDGEMENTS

Funding for this study was provided by South Dakota Agricultural Experiment Station, U.S. Fish and Wildlife Service, and South Dakota Game Fish and Parks. Thank you to Dr. Jonathan Jenks for his direction, sharing of knowledge, and support throughout this study. Additionally, I would like to thank Dr. Jonathan Lundgren for his knowledge of neonicotinoids and support of me as a person as well as a student. Finally, I would like to thank Dr. Dorothy Wells for being a great role model, sharing her knowledge with me, as well as reassuring me that it would "all come together"!

A huge thank you to the PhD students Brandi Felts and Eric Michel for their direction and sharing of knowledge at the SDSU Wildlife Facility and for Eric's help with edits of portions of this thesis. A huge thank you to the graduate students in the office who were *always* supportive and willing to drop what they were doing to assist with a question or move some smelly deer, especially to my best friend Bailey Gullikson, the best desk buddy Alex Rosburg, always supportive Kaylee Faltys, Aaron Suehring (so thankful for you moving smelly deer all the time!), Katherine Moratz, Chad Kaiser, Randy Johnson, Sarah Nevison, Bren Parr, and Ben Don you guys brightened every day! I would also like to thank the folks at Blue Dasher Farm: Claire LaCanne for your support in tough decisions and for the company on late lab nights, Jacob Pecenka for your friendship and willingness to help, and Mike Bredeson for your knowledge of neonicotinoids and willingness to take lots of time to help me understand. I would also like to say a special thanks to those that woke up early and helped move deer through a chute especially Spencer Carstens, Austin Wieseler, Justin Jensen, Corey Lee, Bri Becher, Cassie Auxt, Bailey Gullikson, Katherine Moratz, Claire LaCanne, Jacob

Pecenka, Mike Bredeson, Jon Jenks, Brandi Felts, Jon Lundgren, Eric Michel, and Michele Mucciante. I would also like to thank the technicians that worked tirelessly at the pens over constantly changing schedules, Bri Becher, Corey Lee, John Christenson, Austin Wieseler, and Justin Jensen as well as the countless volunteers that assisted with behavioral observations.

I would also like to thank Dr. Michele Mucciante for her care for the deer, her willingness to come out to the pens at the drop of a hat, and for her administration of drugs to all the deer at the facility. I would also like to thank Dr. David Knudsen for his advice and so many necropsies of deer- you made a stinky job an enjoyable one! I would also like to thank the Lundgren family who fed and housed me during long lab nights, it was most appreciated and always delicious and comfortable!

Finally, I would like to thank my family for their unwavering support and encouragement. Especially to my Grandma Barb for allowing me a place to make this thesis my priority and to Grandma Kathy for your financial support. You have given me the roots of where I am today and I am so thankful. And finally, to my dear feline friend Willard- you have comforted me throughout this entire journey and I am so thankful. I would have achieved very little without these wonderful people in my life- I am forever grateful.

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ABSTRACT

ASPECTS OF WHITE-TAILED DEER ECOLOGY AND MANAGEMENT: EFFECTS OF IMIDACLOPRID AND AN EVALUATION OF MANAGEMENT AT GREAT SWAMP NATIONAL WILDLIFE REFUGE.

ELISE HUGHES BERHEIM

2018

White-tail deer (*Odocoileus virginianus*) are an economically and recreationally important species throughout their range, and understanding their ecology to implement beneficial management techniques is invaluable. This thesis focused on two studies 1) the effect of Imidacloprid on adult female and fawn physiology and 2) an evaluation of techniques to monitor white-tailed deer at Great Swamp National Wildlife Refuge (GSNWR). Imidacloprid, a widely-used pesticide, has been linked to lethal and sub-lethal effects in insects and small vertebrates, however, no research has been conducted on white-tailed deer. Twenty captive pregnant adult female and subsequent fawns housed at the South Dakota State University Wildlife Farm were separated into four pens and given different concentrations of aqueous Imidacloprid (Control 0 ng/L, Low 1,500 ng/L, Moderate 3,000 ng/L, and High 15,000 ng/L) in 2015 and 2016 (May-October). Data collected included water consumption rates, Free Triiodothyronine (FT3) and Free Thyroxine (FT4) hormones, behavioral observations, organ weights, jawbone lengths, and Imidacloprid concentrations in organs. Our results indicated that 1) Imidacloprid was more environmentally prevalent than anticipated, 2) fawn spleen Imidacloprid concentrations showed a negative effect on fawn survival (p = 0.00005), FT4 levels (p =(0.009), and size (p < 0.05), and 3) Imidacloprid presence decreased adult

female and fawn activity. The second portion of this study focused on an evaluation of GSNWR techniques for estimating deer population size to aid in future refuge management. Two separate density surveys (Distance Sampling Spotlight Survey and Baited Trail Camera Survey) were completed in 2015 and 2016 at GSNWR and adjacent Lord Stirling Park Somerset County Environmental Education Center (SCEEC). Results indicated that GSNWR population was well below its management goal of 7.7 deer/km² due to a hemorrhagic disease outbreak that reduced the population by an estimated 57%. Density differences were great between the GSNWR and SCEEC properties most likely due to differences in harvest strategies. From these density estimates, historical refuge data, and the literature, a population model was created and predicted that the refuge would reach 7.7 deer/km² by the 2019 fawning season. The model allows for harvest numbers to be incorporated to monitor and manage future population.

CHAPTER ONE: EFFECTS OF NEONICOTINOID INSECTICIDES ON PHYSIOLOGY AND REPRODUCTIVE CHARACTERISTICS OF CAPTIVE FEMALE AND FAWN WHITE-TAILED DEER

1.0 Abstract

Over the past decade, morphological and developmental abnormalities were documented in elk (Cervus elaphus) and white-tailed deer (Odocoileus virginianus) in west-central Montana. Hypotheses to explain these anomalies include contact with endocrine disrupting pesticides, including the neonicotinoid imidacloprid. Effects of imidacloprid were evaluated experimentally at the South Dakota State University Wildlife and Fisheries Captive Facility where adult white-tailed deer females and their fawns were administered 1-4 doses of aqueous imidacloprid (an untreated control, and low 1,500 ng/L, medium 3,000 ng/L, and high 15,000 ng/L levels of imidacloprid). Thyroid hormone function, behavioral responses, and skull and jawbone measurements of fawns (0-6 months) were compared among treatments. Additionally, enzyme-linked immunosorbent assays (ELISA) were used to determine the level of imidacloprid in liver, spleen, genital, and brain tissue samples of adult females and fawns. Results indicated that control deer consumed more water than the treatment groups, which may indicate that deer were avoiding the insecticide. It also was found that imidacloprid was present in the organs of our control group indicating that our experiment was contaminated from imidacloprid in the local environment. This finding changed our study from treatment dependent, to a post-hoc assessment of the relationship between imidacloprid presence in organs and physiological responses. We found that fawns that died during our experiment had significantly greater spleen concentrations of imidacloprid than those that survived.

Additionally, imidacloprid levels in the spleen were negatively correlated with fawn thyroxine levels, jawbone lengths, body weight, and organ weight. Moreover, adult female imidacloprid levels in the genitals were negatively correlated with organ weights. Finally, behavioral observations indicated that imidacloprid levels in spleens were negatively correlated with activity levels in adult females and fawns. Results indicate that imidacloprid can have direct effects on white-tailed deer when administered at field– relevant doses.

2.0 Introduction

Neonicotinoids are broad-spectrum insecticides that act on the central nervous system of insects (Meijer et al. 2014). In developed countries, neonicotinoids are predominantly used as seed dressings on major field crops, and are additionally used as sprays in crop production, in managing household pests, and deterring pests on domesticated animals (Goulson 2013). Neonicotinoids derive their toxicity from agonistically binding to nicotinic acetylcholine receptors (nAChRs) on the post-synaptic membrane and firing nerve impulses in a manner that is uncontrollable and uninterrupted (Tomizawa and Yamamoto 1992, Buckingham et al. 1997, Matsuda et al. 2001, Rose 2012, Van Dijk et al. 2013, Sánchez-Bayo 2013, Goulson 2013). In insects, this continual nerve impulse causes sub-lethal effects of impaired navigation and feeding (Desneux et al. 2007, Lundgren 2017) as well as producing tremors, hyperactivity, and convulsions that can eventually lead to death. Neonicotinoids have a higher affinity for insect nAChR than for those in vertebrate species (Abou-Donia et al. 2008, Goulson 2013) because mammals have different receptor subtypes than invertebrates; insect receptors are more susceptible to irreversible binding of neonicotinoids (Gupta et al. 2014). Neonicotinoids were first developed in the 1990s (Tokumato et al. 2013), gained popularity from 2003-2011 (Douglas and Tooker 2015), and are now the most widely used pesticide in the world (Jeschke et al. 2011).

Popularity of neonicotinoids is due to their advertised high toxicity to insects and low toxicity to vertebrates (Goulson 2013). Additionally, neonicotinoids have gained popularity by their ability to systemically protect plants while reducing application inputs for farmers (Jeschke et al. 2011). In 2014, over 3.3 million kg of neonicotinoids (including acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid, and thiamethoxam) were used in the United States (excluding Hawaii and Alaska) on pasture hay, alfalfa, orchards, grapes, rice, vegetables, fruit, cotton, wheat, soybeans, corn, and other crops (USGS 2017). In South Dakota, more than 94% of U.S. corn and 50% of U.S. soybeans (Stockstad 2013) are treated with one of the three neonicotinoids: clothianidin, imidacloprid, or thiamethoxam (Main et al. 2014; Seagraves and Lundgren 2012; Bredeson and Lundgren 2015).

Neonicotinoids are widely found in the environment for numerous reasons. First, only a small quantity (2-20%) of the seed-coated insecticide is absorbed by the developing plant; the remainder is released into the environment through leaching, drainage, run-off, or snowmelt (Mason et al. 2013 and Maloney et al. 2017). Because neonicotinoids are highly water soluble (Morrissey et al. 2015), they are prevalent in diverse water bodies in the United States, Canada, Australia, Europe, and Asia (Maloney et al. 2017). Moreover, under the right conditions, neonicotinoids can persist in the soil, sometimes for many years (Goulson 2013). Finally, untreated plants associated with

cropland are often contaminated by neonicotinoids due to their systemic nature (Mogren and Lundgren 2016). The widespread use of neonicotinoids provides numerous opportunities for exposure to non-target, beneficial species via the water, soil, and contaminated plant tissues.

In addition to their documented effects on beneficial insects, neonicotinoids adversely affect non-target vertebrates as well, including rats (*Rattus norvegicus*: reduced sperm production, reduced offspring weight, increased abortions, skeletal abnormalities, thyroid lesions, atrophy of retina, reduced weight gain of offspring, oxidative stress, and neurobehavioral deficits), mice (*Mus musculus*: suppressed cell-mediated immune response and prominent histopathological alterations in spleen and liver), rabbits (*Sylvilagus* sp: increased frequency of miscarriage and premature births), red-legged partridges (*Alectoris rufa*: reduced adult and chick survival, fertilization rate, and immune response), Nile tilapia (*Oreochromis niloticus*: extensive disintegration of testicular tissue and changes to gonads), Medaka (*Oryzias latipes*: juvenile stress led to ectoparasite infestation), and black-spotted pond frogs (*Rana nigromaculata*: DNA damage at very low concentrations) (Gibbons et al. 2014). To our knowledge, no information is available on potential effects on large mammals, such as white-tailed deer (*Odocoileus virginianus*).

White-tailed deer are recreationally and economically important to the United States and to South Dakota. In 2016, 9.2 million hunters participated in large game (including white-tail deer) hunting in the U.S (U.S. Fish and Wildlife 2017). A survey in 2002 indicated that white-tailed deer hunting alone accounts for over 300,000 jobs and \$2.9 billion in federal taxes in the United States (LaBarbera 2002). In South Dakota, deer license sales account for three times more than the combined license sales of any other game species (South Dakota Department of Game, Fish and Parks 2017) and hunting supports over 3,000 jobs and \$23.3 million in taxes (LaBarbera 2002). In 2014, approximately \$4.8 million in license revenue was provided for conservation efforts in South Dakota (South Dakota Department of Game, Fish and Parks 2017). Furthermore, deer hunting provides an important American traditional recreational opportunity (LaBarbera 2002). Besides economic and traditional value, white-tailed deer are a keystone species for the environment (Rooney 2001). Research has shown that whitetailed deer have substantial ecological impacts on vegetation, especially when overpopulated which can lead to a loss of forage and coverage for other species in that ecosystem (Waller and Alverson 1977).

Over the past decade, morphological and developmental abnormalities have been documented in white-tailed deer in west-central Montana. Of 254 male deer of various ages, 67% showed genital developmental abnormalities such as mispositioned and undersized scrota and ectopic testes (Hoy et al. 2002); these abnormalities were documented for accident-killed and injured cervids (Hoy et al. 2002). Hoy et al. (2002) suggested that genital anomalies could be caused by endocrine disrupting pesticides but stated that based on the information available, no cause and effect could be justified. In addition, from 2000 to 2009, brachygnathia superior (i.e., mandibular prognathia or underbite) increased from 0% to 70% for white-tailed deer that were collected from westcentral (accident killed) and throughout (hunter harvested) Montana (Hoy et al. 2011). Underbite is a characteristic of congenital hypothyroidism, which has been documented in South Dakota (Zimmerman et al. 2004), and is nearly always associated with fetal thyroid hormone function (Hoy et al. 2011), but the cause has not been empirically determined for this observation.

We hypothesized that imidacloprid would have sub-lethal and potentially lethal effects on adult female and fawn white-tailed deer. We predicted that adult females, especially in the high treatment group, would have reduced Free Triiodothyronine (FT3) and Free Thyroxine (FT4) levels, presence of imidacloprid in milk, and reduced activity associated with exposure to imidacloprid. We also predicted that fawns exposed to imidacloprid at relatively high treatment levels would have abnormal genital organs, lowered FT3 and FT4 levels, reduced activity, and a high prevalence of underbite.

3.0 Study Area

Our research study was conducted at the South Dakota State University Wildlife Captive Research Facility in Brookings County, South Dakota (44°20'N, 96°47'W). This facility housed white-tailed deer (beginning in about 1998) on 4 ha; the facility is double fenced with 3-m high woven wire. The facility is situated adjacent to agricultural fields normally planted to corn or soybeans, and is surrounded by a shelterbelt of trees. Mean annual temperatures were 7.4° C (ranged from -28.8° C and 34.4° C) and 7.8° C (-35° C to 32.8° C) in 2015 and 2016, respectively. Additionally, daily annual precipitation was 0.18 cm (0.03 cm to 5.2 cm) and 0.2 cm (from 0.3 cm to 7 cm) in 2015 and 2016 respectively. Finally, daily annual snowfall was 0.3 cm (0.3 – 17.8 cm) and 0.3 cm (0.3 – 11.4 cm) in 2015 and 2016 respectively (NOAA 2017).

4.1 Experimental Design

Our experiment was conducted in 2015 and 2016 at the SDSU Captive Wildlife Research Facility, Brookings, South Dakota. Twenty adult female, white-tailed deer were randomly selected for the experiment and bred; parturition occurred in May and June of each experimental year. Adult females were separated into four treatments (care was taken to separate adult females so that age and weight were uniformly distributed): control (n=4), low (n=4), moderate (n=5), and high (n=7) (the moderate and high treatment groups had a larger sample size to reduce the standard error for our response variable). Deer were housed in pens of similar size (control = 130 m²/deer, low = 175 m²/deer, moderate = 123 m²/deer); however, the high treatment pen was larger (high pen in 2015 was 112 m²/deer and in 2016 the pen was enlarged to 165 m²/deer) due to the increased number of adult females and fawns housed. All deer were fed rations that included soy hulls, shelled corn, and alfalfa hay *ad libitum*.

Adult females were administered aqueous imidacloprid (Product # 37984, Sigma Aldrich St. Louis, MO) from May until October to mimic free water availability within the Dakotas. We added 0 ng/L, 1,500 ng/L, 3,000 ng/L, and 15,000 ng/L of imidacloprid to the control, low, moderate and high treatments, respectively. The low and moderate concentrations were similar to wetland levels found in groundwater in Wisconsin (detected in 24% of the groundwater sample and ranged from 260-3340 ng/L), however they are greater than the levels found in rural streams in Iowa (detected in 23% of streams sampled and ranged from <2-42.7 ng/L) or in Canadian (Saskatchewan) wetlands (detected in 12% of wetlands and ranged from 7.1-256 ng/L) (Giorio et al. 2017). Our high treatment was intended to invoke an effect and therefore, was much greater than

documented in free water. Deer were provided with a 60.6 L tub that contained 37.8 L of water treated with the appropriate amount of imidacloprid depending on the group (control, low, moderate, high). Deer consumed the water treated with imidacloprid *ad libitum*. Water levels were checked daily and refilled with the appropriate imidacloprid treated water when empty or less than 3 cm from the bottom (every 1-2 d) of containers. When refilling occurred, each tub was rinsed thoroughly and excess water was poured into 189 L tubs provided by the SDSU Environmental Health and Safety office.

Fawns born to adult females in the study were included in our experiment. On the day of parturition, each fawn was handled minimally with gloves to determine body mass and sex; fawns also were fitted with ear tags. To mimic natural water availability, fawns were not prevented from consuming the imidacloprid in water. Facilities and techniques for research were approved by the Institutional Animal Care and Use Committee (IACUC number 15-055A) and followed guidelines by the American Society of Mammologists (Sikes et al. 2016).

4.2 Solution Consumed

During experiments, water tubs housing aqueous imidacloprid were weighed daily to determine the volume of water consumed per group. Analysis of variance (ANOVA) was performed to compare water consumption between treatment and control groups with date used as a covariate. To detect imidacloprid concentrations as the imidacloprid water was consumed, a 3-d experiment was conducted. On day 1, the appropriate treatment or control group concentrations were created in five, 63 L galvanized tubs. On day 2, 50% of the water was removed from all tubs. On the third day, nearly all water was removed from the tubs, leaving only enough water to coat the bottom of tubs. Samples (15 mL) were collected daily from each tub. This procedure mimicked water level reductions due to deer consumption. Imidacloprid samples were analyzed using ELISA (enzyme-linked immunosorbent assay; Abraxis, Warminister, PA; See Section 4.7 for procedures).

4.3 Collection of blood samples

Blood samples were collected from adult females and fawns in treatments using BD Vacutainer Serum tubes (Becton, Dickinson, and Company, Franklin, NJ). We collected up to 12 mL of blood from the saphenous vein approximately monthly during treatments while deer were held in a chute (Priefert Wildlife Equipment Deer Chute; Priefert®, Mount Pleasant, TX). We collected blood samples (1-10cc from the saphenous or jugular) from fawns twice; 1 wk after parturition and at approximately 5 mo of age. Blood samples were refrigerated until processed to extract serum (1 h to 2 d). Upon reaching the lab, blood samples were centrifuged (Ultra-8V; LW Scientific, Lawrenceville, GA) for 15 min at 280× g to separate serum for hormone testing of FT3 (free triiodothyronine) and FT4 (free thyroxine).

FT3 and FT4 thyroid hormones reflect the ability of the deer to utilize body fat reserves, regulate basal metabolic rate, and control thermal regulation (Bergman 2014). Serum from blood samples were transferred to labeled 1.5 mL microcentrifuge tubes (BrandTech® Scientific Inc., Essex, CT), sealed, and frozen at -20° C. These samples were then overnighted to the Diagnostic Center for Population and Animal Health at Michigan State University (Lansing, MI) for FT3 and FT4 testing. These assays were performed with commercially available solid-phase radioimmunoassay kits (FREE T3 Solid Phase Component System and Free T4 Solid Phase Component System, MP Biomedicals Diagnostics Division Orangeburg NY 10962). The volumes of sample, assay standards, and radioligand were used according to the manufacturer's protocol. Incubation times for free T3 and free T4 assays were 2.5 h and 1.5 h, respectively, at 37° C.

4.4 Behavioral Observations

Focal sampling behavioral observations were collected on treatment and control groups. Behaviors included eat, lay, lay/groom, lay/ruminate, stand/ruminate, run, stand, stand/groom, stand/nurse, and walk and additionally for fawns the behaviors lay/curl and lay/sleep also were recorded. Observations were conducted in 1 h blocks using an ethogram (Altmann 1974). During time blocks, occurrences of behaviors were tallied and the duration of each behavior (in s) was recorded. Observations occurred between 6:00 and 16:00. In each session, an adult female or fawn was randomly chosen (without replacement) from each treatment and control group, and sessions were conducted until at least 50% of the does and fawns were observed in each treatment per month. A total of 257 hour observations was collected for adult females and fawns in 2015 and 2016, however, for analyses, the only observations used were those collected most recently prior to death (n=28 for 2016 fawns and n=21 for adult females). Not all fawns that died prematurely were not observed in trials.

4.5 Necropsies

All deer in the experiment (adult females and fawns) were euthanized and subsequently necropsied using IACUC approved protocols. Fawns were euthanized at the end of each field season (October 2015 and 2016) and adult females were euthanized at the completion of the study (October 2016). Adult females and fawns were first tranquilized using xylazine (Bayer, Englewood, Colorado) and telezol (Zoetis, Parsippany-Troy Hills, New Jersey) when held in a Priefert deer chute and, once immobilized, were euthanized using euthanasia solution (MWI Veterinary Supply, Boise, Idaho) according to manufacturer's suggested dosage. Once does and fawns were euthanized, they were frozen at -20°C. All fawns and does in the experiment were necropsied at the South Dakota Animal Disease Research and Diagnostic Laboratory, South Dakota State University, Brookings, South Dakota.

Necropsies were performed by Dr. David Knudsen (assisted by E. Hughes Berheim). Liver, brain, spleen, and genital organs were extracted, weighed, and 2.54 cm³ samples were collected. Additionally, we collected fawn jawbones to determine length. Organ samples were then frozen at -20° C until they could be analyzed using ELISA.

4.6 ELISA Testing

Imidacloprid levels were determined for each organ collected. Brain, liver, spleen, and genital samples were removed from the freezer and a portion of each organ (0.5 – 0.75 g) was minced using a sterilized scalpel, and placed into a polypropylene micro centrifuge tube. Water was added to the tube at a sample to water ratio of 1g:1mL. Each mixture was shaken using a vortex (Thermo Scientific), heated in an 80° C water bath for 10 min, and frozen at -20° C. Frozen mixtures were thawed and centrifuged (Centrifuge 5424, Eppendorf) at 21,130 g for 1 min. The liquid was extracted and placed into separate micro centrifuge tubes; remaining solids in the organ samples and remaining liquid was refrozen. Liquid samples were vortexed and a 25 μ L portion was extracted and placed into a separate microcentrifuge tube. The excess liquid also was stored frozen. The remaining liquid was mixed with 25 μ L of water, vortexed for 5 s, and centrifuged for 2 s in preparation for the ELISA assay.

All samples were read at 450 nm using a microplate reader (uQuant, Biotek Instruments, Winooski, VT). Each plate had at least two standard curves of purified imidacloprid (Product number: 37894 SIGMA-ALDRICH, St. Louis, MO, USA). In preparation for the standard curve on each plate, samples from negative adult females were mixed together to account for the matrix effect of the organs and a stock solution of imidacloprid was created at 0.0, 0.03, 0.06, 0.13, 0.25, 0.5, 1.0, and 2.0 ppb. The standard curve on the ELISA plate contained 25 μ L control organ in solution with 25 μ L of the stock solution of imidacloprid, creating eight wells with concentrations that comprised one standard curve. Since our experiment was contaminated, we couldn't use the control organs in our standard curve, therefore we took a 25 μ L sample from the lowest ELISA available to quantify our results.

4.7 Analysis

Data collected in experiments were analyzed using Systat 13 (Systat Software Inc., San Jose, CA). Male and female fawn organ concentrations for those fawns that survived versus those that died were compared using a t-tests. ELISA organ results indicated that there was contamination in our control group. As a consequence, ordinary least square (OLS) linear regression was used to assess relationships between imidacloprid concentrations in all organ samples and the physical responses of birth weight, fawn age, FT3, FT4, jawbone length, organ weights; alpha was set at P = 0.05 to support significance for these analyses.

Data collected on behavioral observations for adult females and fawns were analyzed separately but combined over observation period (morning and afternoon). Additionally, behaviors recorded were combined into 10 behaviors based on the most frequent behavioral occurrence. Furthermore, we separated deer into three groups (high, moderate, and low) based on organ imidacloprid concentrations. Finally, we used Chisquare tests to determine significant differences among behaviors observed in high, moderate, and low imidacloprid groups for adults and fawns. If Chi-square tests were significant, we used confidence intervals (90%) to assess which behaviors differed among groups (high, moderate, and low).

5.0 *Results*

5.1 Doe and Fawn Survival

A total of 24 and 39 fawns was born in 2015 and 2016, respectively. In 2015, 12 of the fawns were born in August and September due to late breeding. In 2016, a control female died (#19) and was replaced with another adult female, totaling 21 adult females in our experiment. Fawn and adult female survival decreased over the two field seasons: survival of fawns was 75% and 62% in 2015 and 2016, respectively. Of 20 adult females in 2015, 0% died (100% survival) and in 2016 19% of 21 adult females died (n = 4, 81% survival) (Table 1). Survival of fawns did not differ (p > 0.05) between 2015 and 2016.

Additionally, sample size for adult females was too small to distinguish a significant difference in survival between the two field seasons.

5.2. Imidacloprid Solution Consumption

Water consumption rates in 2015 and 2016 were monitored and daily consumption was recorded. In 2015, there were significant differences in adult female consumption among treatments ($F_{3, 436} = 12.01$, p = 0.01), sample dates ($F_{5, 436} = 37.09$, p = 0.01), and in the interaction between treatment and date ($F_{15, 436} = 2.22$, p = 0.01). In 2016, there also were significant differences in adult female consumption of water among treatments ($F_{3, 555} = 16.69$, p = 0.01), sample dates ($F_{5, 555} = 22.06$, p = 0.01), and in the interaction between treatment and date ($F_{15, 555} = 2.19$, p = 0.01). In 2015, when the control was removed from the analysis, date was still significant ($F_{5, 327} = 21.48$, p = 0.01) relative to consumption; however, water consumption per adult female was similar across treatments ($F_{2, 327} = 0.60$, p = 0.55), indicating the control group consumed significantly more water than the treatment groups. In 2016, when excluding the control group, the high treatment group consumed less water per adult female than the low and moderate groups ($F_{2, 421} = 12.83$, p = 0.01) even though consumption of water increased throughout the field season ($F_{5, 421} = 14.25$, p = 0.01) (Table 2).

5.3 Necropsy Data

Organ weights were collected from adult females and fawns and jawbone measurements were collected solely from fawns. Adult females had mean organ weights of 159 ± 8 g for brain, 809 ± 104 g for liver, 388 ± 41 g for spleen, and 87 ± 28 g for genitals. Fawn mean organ weights of 106 ± 3.9 g for brain, 413 ± 37 g for liver, 102 ± 11.4 g for spleen, and 6 ± 0.9 g for genitals. Female fawn mean organ weights were 96 ± 6 g for brain, 342 ± 55 g for liver, 95 ± 18 g for spleen, and 3 ± 0.6 g for genitals. Male fawn mean organ weights were 115 ± 5 g for brain, 479 ± 48 g for liver, 109 ± 14 g for spleen, and 9 ± 1 g for genitals (Table 3). Average jawbone length results were 13.8 ± 0.4 cm.

5.4 ELISA Results

Imidacloprid concentrations (ng/L) for organ samples from adult females and fawns were determined by the ELISA. ELISA results indicated imidacloprid was found in the control group organs (Table 4), indicating that our treatments were contaminated. This changed our focus from separating ELISA results by treatments to viewing the results relative to concentration of imidacloprid. Mean imidacloprid values in organs for all adult females were 0.42 ± 0.07 ng/g for liver, 0.06 ± 0.05 ng/g for brain, 0.11 ± 0.04 ng/g for spleen, and 0.69 ± 0.05 ng/g for genital (Table 4). Mean imidacloprid values in organs for female fawns were 0.42 ± 0.06 ng/g for liver, 0.03 ± 0.02 ng/g for brain, 0.21 ± 0.05 ng/g for spleen, and 0.26 ± 0.04 ng/g for genital (Table 4). Mean imidacloprid values in organs for male fawns were 0.55 ± 0.07 ng/g for liver, 0.05 ± 0.02 ng/g for brain, 0.19 ± 0.04 ng/g for spleen, and 0.15 ± 0.03 ng/g for genital (Table 4).

5.5 Analyses

Due to the contamination in our study, we used linear regressions on organ (liver, brain, spleen, and genitals) imidacloprid values and physical responses to determine relationships between imidacloprid concentrations the physiological responses in deer. Physical responses include fawn survival, fawn birth weight, fawn body weight, fawn age, adult female and fawn FT3 and FT4 concentrations, adult female and fawn organ weights, and fawn jawbone lengths. Spleen concentrations of imidacloprid were significantly higher ($T_{59} = 2.76$, p = 0.007) in fawns that died compared to the fawns that survived. Upon looking at the data, an outlier of 1.49 ng/g of spleen tissue (mean of data with outlier 0.20, range 0-1.49, mean of data without outlier 0.18, range 0-0.91 ng/g of tissue) was found. Additionally the fawn that housed this high spleen imidacloprid concentration survived, which was not representative of the sample and therefore we removed the outlier; the revised analysis also was significant (T (58) = 4.36, p = 0.00005) (Figure 1). We excluded the outlier for the rest of the analysis. Birth weight was not correlated with imidacloprid levels in any of the organs evaluated (Table 5). Fawn body weight at death were negatively correlated with imidacloprid levels in the spleen ($F_{1,55}$ = 8.22, P = 0.005) and genital organs ($F_{1.56} = 4.26$, P = 0.04) (Table 5, Figure 2 and 3). Fawn age at death was correlated with imidacloprid levels in the spleen ($F_{1,57} = 10.5$, P = 0.0019, Figure 4) but not in any of the other organs evaluated (Table 5). Adult female FT3 and FT4 values were not correlated with imidacloprid levels in organs (Table 5). Fawn FT3 values were not correlated with imidacloprid concentrations in organs; however, FT4 values in fawns were negatively correlated ($F_{1,39} = 7.48$, P = 0.0092, Table 5; Figure 5) with spleen imidacloprid concentrations. Adult female organ weights were negatively correlated with imidacloprid concentrations in genitals ($F_{1,19} = 5.00$, P = 0.04)

(Figure 6) but not with other organ levels evaluated. Fawn organ weights were negatively correlated with spleen ($F_{1,57}$ = 8.78, P = 0.0044; Figure 7) and genital ($F_{2,54}$ = 5.55, P = 0.021; Figure 8) (Table 5) imidacloprid concentrations. Fawn jawbone length was negatively correlated with imidacloprid values in the spleen ($F_{1,57}$ = 9.98, P = 0.002; Figure 9) but not with other organ concentrations (Table 5).

Because imidacloprid concentrations in spleen was correlated with fawn survival, we used the spleen imidacloprid concentrations to quantify potential effects on deer behavior. Adult female (n=21) imidacloprid concentrations in spleen were separated into low (n=12, range=0), moderate (n=4, range 0.056-0.224), and high groups (n=5, range= 0.248-0.909); groups were then compared based on behavioral observations (all spleens that had 0 ppb concentration were placed in the low group). The low imidacloprid group differed (90% CI) from the high group in the behaviors eat (groups; high = 2.4%, low = 6%), lay (high = 27%, low = 19%), lay/groom (high = 7%, low = 3%), stand/ruminate (high = 1%, low = 2%), run (high = 1%, low = 5%), and stand/groom (high = 8%, low = $\frac{1}{2}$ 5%) indicating that adult deer in the low group had higher activity levels than those in the high group. The moderate group also differed from the low group in the behaviors eat (group; moderate = 10%, low = 6%), lay (moderate = 4%, low = 19%), lay/ruminate (moderate = 2%, low = 5%), stand/ruminate (moderate = 1%, low = 2%), run (moderate = 1%, low = 5%), stand (moderate = 34%, low = 23%), stand/groom (moderate = 13%, low = 5%), and stand/nurse (moderate = 0%, low = 2%); indicating variation in results between the two groups (Table 6).

Fawn spleen concentrations of imidacloprid (n=38) also were placed in a low (n=20, range=0), moderate (n=9, range= 0.053-0.121), and high (n=9, range=0.148-

0.786) group and compared relative to behavioral observations. The low group differed (90% CI) from the high group in the behaviors lay (group; high = 43%, low = 24%), run (high = 0%, low = 4%), stand (high = 16%, low = 22%), stand/groom (high = 2%, low = 6%), and walk (high = 9%, low = 15%); indicating that the high group was less active than the low group. The moderate group also differed from the low group in the behaviors eat (group; moderate = 12%, low = 8%), lay/curl (moderate = 1%, low = 6%), and lay/groom (moderate = 15%, low = 8%); overall indicating variation in results between the two groups (Table 6).

6.0 Discussion

Our study provides the first overview of effects of imidacloprid on white-tailed deer. First, our experiment provides support that imidacloprid is more prevalent in the environment than we had anticipated. Additionally, we documented that deer in our experiment avoided imidacloprid-contaminated water. Moreover, we discovered that fawns that died during our experiment had greater concentrations of imidacloprid in spleens compared to those that survived. Fawns with relatively high concentrations of imidacloprid in spleen and genital organs also tended to be smaller and less healthy than those with relatively low concentrations of imidacloprid in organs. Finally, our study provides support for reduced activity of adult and fawn white-tailed deer with relatively high concentrations of imidacloprid in spleens.

ELISA results indicated that our control experimental tissues were contaminated with imidacloprid. Potential sources of contamination included seed-treated food and vegetation. Deer were fed soy hulls and a corn, oats, and distiller's mixture *ad libitum*.

Unfortunately, the origin of the soybeans and grains fed to our deer were unspecified. Therefore, it is unknown what specific pesticides were potentially on the feed. However, corn and soybeans are commonly (\geq 94% of U.S. corn, ~50% of U.S. soybeans; Stokstad 2013) coated with one of the neonicotinoid active ingredients; clothianidin, imidacloprid, or thiamethoxam (Main et al. 2014). Additionally, deer in our study would often reach through the fence to browse on outside vegetation. The adjacent fields to the SDSU captive wildlife facility were a matrix of agricultural crops with a corn field about 50 m north of the facility. It is unknown what kind of pesticide was used on the corn, but it is likely that there was a seed treatment of imidacloprid or clothianidin used. In Indiana, neonicotinoid dust was documented to disperse as far as 100 m from the site (Krupke 2017). Imidacloprid from fields could be washed off during rain events and be absorbed by other plants, although this transfer is poorly understood (Bonmatin, 2015, Botias et al. 2016, and Mogren and Lundgren 2016). Therefore, uptake of imidacloprid by vegetation adjacent to the facility is a reasonable possibility.

Water containing imidacloprid was avoided by deer in treatments in our experiment as evidenced by variable concentrations of the neonicotinoid in captive deer. Deer that avoided consumption of treated water likely drank rain water, which was available (up to 0.3 m deep) after storm events during our experiment. Research on Cervid avoidance of imidacloprid is unavailable, but avoidance of imidacloprid has been recorded in red-legged partridge (*Alectoris rufa*) when offered treated seeds (Antia et al. 2014). Other animals detect and avoid toxins in their diets; for example, kudus (*Tragelpahus imberbis*), impalas (*Aepyceros melampus*), and goats (*Capra aegagrus*) *hircus*) in South Africa avoided plants with 5% condensed tannins during the wet season (Cooper and Owen-Smith, 1985), likely due to the astringency of these compounds.

Significantly higher concentrations of spleen imidacloprid levels were found in fawns that died compared to those that survived. The spleen purifies blood and produces white blood cells that fight infection and synthesize antibodies (Mebius et al. 2017). Imidacloprid can reduce the production of spleen lymphocytes (Mohany et al. 2011, Gawade 2013, Mohany 2012), which results in an impaired immune system (Gibbons 2014). Therefore, immune suppression in our fawns caused by imidacloprid likely was a factor in their deaths. Complimentary results were found in the FT4 values that are a precursor to FT3 hormone and is instrumental in regulating basal metabolic rate and thermal regulation in deer (Bergman 2014). FT4 was inversely correlated with imidacloprid in spleens of fawns. Reduced metabolic rate in fawns with relatively high concentrations of imidacloprid likely explain the lower activity documented in captive deer.

Imidacloprid values in brain were low to undocumented, which was surprising considering that the pesticide affects the central nervous system; we hypothesize that this could be due to an inability of the chemical to cross the blood-brain barrier. The California Environmental Protection Agency found that imidacloprid penetrates the blood-brain barrier. However, Gupta et al. (2014) found high imidacloprid quantities in rat liver, kidney, lung, and skin, but concentrations in the brain were low. Additionally, Krieger (2010) noted that the blood-brain barriers in vertebrates block access of imidacloprid to the central nervous system, which reduces toxicity.

Increased imidacloprid levels in spleen and genital tissues were negatively associated with body weight in fawns at the time of death. Fawns had similar birth weights regardless of the level of imidacloprid in their organs. Similarly, in Sprague-Dawley rats, there were no differences in litter size or weight gain in the offspring whether or not mothers were given an intraperitoneal injection of imidacloprid (Abou-Donia et al. 2008). Additionally, Gawade (2013) found no significant difference in weights of imidacloprid exposed Wister rat pups.

FT3 and FT4 results are indicative of basal metabolic rate and thermoregulation (Bergman 2014). Fawn and adult female FT3 values were similar to those reported in other studies, but FT4 results were elevated compared to previous studies (Watkins 1981, Creekmore et al. 1999, Bishop et al. 2009, and Bergman et al. 2014). We do not believe that this was the result of imidacloprid, as this pesticide decreases thyroid function in rats (Abbassy et al. 2014), Indian wild birds (Pandey et al. 2014), and fish (Priya et al. 2014). Rather, the elevated FT4 values may be due to a combination of pregnancy in adult females, the time of year, and artificial feed. A study by Hamr et al. (2011), found that thyroid hormones of artificially fed deer were elevated compared to deer that consumed natural browse. Additionally, this study also found that hormones were increased in the spring and summer. Further, Bahnak et al. (1981) documented that pregnant, penned deer have elevated levels of thyroid hormones.

As imidacloprid increased in the spleen, we saw that FT4 levels and spleen size decreased. As stated previously, imidacloprid has been shown to decrease FT4 levels in other vertebrates (Abbassy et al. 2014, Pandey et al. 2014, and Priya et al. 2014). Additionally, research on rats has shown that organ weights (specifically liver and spleen) decrease as imidacloprid treatment increases (Vohra et al. 2014, Memon et al.

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2014, and Vohra et al. 2016). From our observations and previous research outcomes, we predict that imidacloprid is suppressing the immune function and size of the spleen.

As imidacloprid increased in the genital organs of fawns and adult females, weights of the genital organs decreased. A study on lab rats by Vohra and Khera (2016) found that as oral consumption of imidacloprid increased, the ovaries became smaller but the uterus increased in size. Additional research has shown that liver and spleen sizes will decrease as imidacloprid increases however, there was not an indication that the genital weight decreased (Vohra et al. 2014, Memon et al. 2014, and Vohra et al. 2016). Consequently, more research is needed to better understand how imidacloprid and other neonicotinoids affect reproductive tissues in mammals.

Behavioral observations indicated that high concentration of imidacloprid in the spleen resulted in less activity in the adult females and fawns. This finding was similar to results on female rats and their offspring that showed significant decreases in grip time as imidacloprid concentrations from intraperitoneal injection increased, an indication of fatigue (Abou-Donia et al. 2008). Rat movement was similarly impaired as imidacloprid (via oral consumption) increased (Memon et al. 2014, Najafi et al. 2009).

7.0 Acknowledgments

Funding for this study was provided by the South Dakota Agricultural Experiment Station and South Dakota Department of Game, Fish and Parks (Study No. 3M5842). Dr. Michele Mucciante provided veterinary assistance for the duration of this study. We thank B. Felts and E. Michel for help with captive deer and for comments on an earlier draft of our manuscript. Others who assisted with captive deer included: S. Carstens, A. Wieseler, J. Jensen, C. Lee, B. Becher, C. Auxt, B. Gullikson, K. Moratz, C. LaCanne, J. Pecenka, and M. Bredeson. I would also like to extend a special thanks to the technicians that worked at the captive facility: B. Becher, C. Lee, J. Christenson, A. Wieseler, and J. Jensen as well as the countless volunteers that assisted with the collection of behavioral observations. Thank you to M. Bredeson, C. LaCanne, and J. Pecenka for help with ELISAs. Finally, a thank you to Dr. D. Knudsen for help with necropsies.

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Table 1. Total number of deer (n= 86 adult females and fawns) used for our experiment in 2015 and 2016 including the sex ratio, range of birth dates, average birth and death weights, and percentage of deaths. One fawn could not be sexed due to parturition difficulties (scored as unknown). Fawn deaths occurred in 2015 and 2016, while adult female deaths only occurred in 2016.

Year	n	Male:Female: Unknown	Birth Date Range	Average Birth Weight (kg) (SEM)	Average Death Weight (kg) (SEM)	% Died
2015	24 fawns	11:13:0	5/19-9/6	3.3 (0.12)	17.2 (2.6)	29% (n=7)
2016	41 fawns	21:19:1	5/16-7/28	3.1 (0.1)	15.7 (1.7)	39% (n=16)
2016	21 adult females	N/A	5/1/06- 5/19/15	N/A	60.1 (1.9)	19% (n=4)

Table 2. Water consumed by deer in treatments in 2015 and 2016 field seasons (May to October). Average liters consumed, average liters consumed daily per doe were recorded for each treatment and control group. Average water consumed daily by fawns is also included in the table as it was used as a covariate in the ANOVA analysis of water consumption between treatment and control groups.

Group	Date	Total Liters Consumed (SEM)	Average Liters Per Day (SEM)	Average Liters Per Doe (SEM)	Average Liters Per Fawn (SEM)
Low	2015	1616.7 (0.61)	12.7 (0.61)	3.3 (0.2)	1.72 (0.1)
Moderate	2015	2345.9 (0.78)	17.5 (0.78)	3.5 (0.2)	2.42 (0.1)
High	2015	2806.4 (1.13)	20.8 (1.13)	3.1 (0.2)	3.98 (0.2)
Control	2015	2574.1 (0.78)	19.5 (0.78)	5.2 (0.2)	4.23 (0.3)
Low	2016	2216.5 (9.7)	17 (0.85)	4.2 (0.2)	2.78 (0.1)
Moderate	2016	2323.2 (8.9)	17.7 (0.78)	3.6 (0.1)	3.89 (0.2)
High	2016	2430.3 (13.2)	19.1 (1.2)	3.3 (0.2)	6.06 (0.3)
Control	2016	2295.2 (8.6)	18.4 (0.77)	4.7 (0.2)	4.81 (0.2)

Table 3. Mean organ (brain, liver, spleen, and genital) weights (g) of adult females and fawns including standard error. Sample sizes are as follows: adult female n=21, fawn n=61 for the brain, spleen, and genital and n=62 for the liver, male fawns n=30 for brain, spleen, genital, and n=31 for the liver, and female fawns n=31.

	Brain (g) (SEM)	Liver (g) (SEM)	Spleen (g) (SEM)	Genital (g) (SEM)
Adult female	161 (8)	1015 (63)	408 (40)	64 (15)
Fawn	105 (3.9)	432 (35)	103 (11)	6 (0.8)
Male fawn	115 (5)	479 (47)	109 (14)	9 (1)
Female fawn	95 (6)	385 (53)	99 (17)	3 (0.6)

Table 4. Average imidacloprid levels in organs (ng of imidacloprid per gram of tissue) liver, brain, spleen, and genital in adult females (AF, n=21), fawns (n=65), female fawns (FF, n=32), and male fawns (MF, n=32) per treatment and control groups. AF, FF, and MF are also separated into averages for died, survived, and all (meaning all AF, FF, or MF in our study).

Age/S ex	Group	Survived/ died	Liver (ng/g) (SEM)	Brain (ng/g) (SEM)	Spleen (ng/g) (SEM)	Genital (ng/g) (SEM)
AF	Contr ol	All	0.351 (0.09)	0.222 (0.22)	0.012 (0.01)	0.388 (0.12)
AF	Low	All	0.133 (0.04)	0	0.077 (0.05)	0.380 (0.11)
AF	Moder ate	All	0.495 (0.18)	0.010 (0.01)	0.111 (0.11)	0.287 (0.08)
AF	High	All	0.590 (0.12)	0	0.188 (0.10)	0.210 (0.06)
AF	All	Died	0.153 (0.04)	0.277 (0.21)	0.030 (0.02)	0.191 (0.13)
AF	All	Survived	0.487 (0.08)	0.003 (0)	0.124 (0.05)	0.330 (0.04)
AF	All	All	0.423 (0.07)	0.055 (0.05)	0.106 (0.04)	0.694 (0.05)
FF	Contr ol	All	0.416 (0.06)	0.058 (0.03)	0.156 (0.04)	0.273 (0.04)
FF	Low	All	0.430 (0.05)	0.053 (0.02)	0.114 (0.02)	0.402 (0.04)
FF	Moder ate	All	0.357 (0.05)	0	0.126 (0.02)	0.174 (0.02)
FF	High	All	0.426 (0.12)	0.008 (0)	0.294 (0.13)	0.222 (0.04)
FF	All	Died	0.443 (0.09)	0	0.268 (0.06)	0.219 (0.03)
FF	All	Survived	0.401 (0.07)	0.044 (0.03)	0.177 (0.08)	0.290 (0.06)
FF	All	All	0.417 (0.06)	0.028 (0.02)	0.210 (0.05)	0.264 (0.04)
MF	Contr ol	All	0.681 (0.10)	0.065 (0.02)	0.223 (0.03)	0.102 (0.03)
MF	Low	All	0.350 (0.04)	0	0.037 (0.01)	0.168 (0.05)
MF	Moder ate	All	0.566 (0.08)	0.044 (0.02)	0.252 (0.07)	0.148 (0.04)
MF	High	All	0.532 (0.09)	0.057 (0.04)	0.176 (0.06)	0.157 (0.03)
MF	All	Died	0.654 (0.08)	0.006 (0)	0.489 (0.07)	0.259 (0.04)
MF	All	Survived	0.518 (0.08)	0.057 (0.03)	0.116 (0.03)	0.115 (0.03)
MF	All	All	0.553 (0.07)	0.046 (0.02)	0.193 (0.04)	0.146 (0.03)
Fawn	All	Died	0.528 (0.04)	0.002 (0)	0.342 (0.03)	0.232 (0.02)
Fawn	All	Survived	0.463 (0.05)	0.051 (0.02)	0.144 (0.04)	0.200 (0.03)

	Imidacloprid Concentration								
Physical Responses	Brain	Liver	Spleen	Genital					
Birth Weight	$F_{1,60} = 0.04, P = 0.83$	$F_{1,60} = 0.25, P = 0.61$	$F_{1,58} = 1.25, P = 0.26$	$F_{1,59} = 0.08, P = 0.77*$					
Fawn Body Weight @ Death	$F_{1,57} = 0.98, P = 0.32$	$F_{1,56} = 0.35, P = 0.55$	$F_{1,55} = 8.22, P = 0.0058*$	$F_{1,56} = 4.26, P = 0.04$					
Fawn Age (in Days)	$F_{1,59} = 1.78, P = 0.18$	$F_{1,60} = 0.0008, P = 0.97$	$F_{1,57} = 10.5, P = 0.0019*$	$F_{1,58} = 1.71, P = 0.19$					
AF FT3	$F_{1,19} = 2.96, P = 0.10$	$F_{1,19} = 0.04, P = 0.85$	$F_{1,19} = 1.89, P = 0.18$	$F_{1,19} = 0.04, P = 0.83$					
AF FT4	$F_{1,19} = 4.1, P = 0.06$	$F_{1,19} = 0.09, P = 0.76$	$F_{1,19} = 1.30, P = 0.27$	$F_{1,19} = 0.57, P = 0.46$					
Fawn FT3	$F_{1,39} = 0.41, P = 0.52$	$F_{1,39} = 2.9, P = 0.09$	$F_{1,38} = 0.74, P = 0.39$	$F_{1,39} = 0.20, P = 0.65$					
Fawn FT4	$F_{1,40} = 0.01, P = 0.90$	$F_{1,40} = 0.0002, P = 0.98$	$F_{1,39} = 7.48, P = 0.009*$	$F_{1,40} = 0.017, P = 0.89$					
AF Organ Weights	$F_{1,19} = 0.04, P = 0.84$	$F_{1,19} = 1.15, P = 0.3$	$F_{1,19} = 0.29, P = 0.6$	$F_{1,19} = 5.0, P = 0.04*$					
Fawn Organ Weights	$F_{1,59} = 2.42, P = 0.12$	$F_{1,60} = 0.10, P = 0.75$	$F_{1,57} = 8.78, P = 0.004*$	$F_{1,58} = 5.55, P = 0.02*$					
Fawn Jawbone Length	$F_{1,59} = 1.5, P = 0.22$	$F_{1,60} = 0.11, P = 0.73$	$F_{1,57} = 9.98, P = 0.002*$	$F_{1,58} = 2.38, P = 0.12$					

Table 5. Results of regression analyses for imidacloprid concentrations in organ samples and physical results: birth weight, fawn body weight, fawn age, FT3 and FT4, organ weights, fawn jawbone length. P-values were considered significant when < 0.05.

*Indicates p-values that are significant and indicates a negative correlation so as imidacloprid increases the physical response decreases.

Table 6. Behavioral observations closest to individual adult female (AF: n=21) and fawn (n=38) deaths (time ranged from 1 week to 2 months) were compared to their spleen imidacloprid concentrations. Not all fawns had observations collected as 1) fawn observations were only collected in 2016 and 2) some fawns died prior to an observation being completed. Behavioral observations are separated into three groups (low, moderate, high) according to spleen organ concentrations (with the high group having the greatest imidacloprid levels and the low group having the lowest). Behavioral observations eat, lay, lay/groom (lay/grm), lay/ruminate (lay/rum),

stand/ruminate (sta/rum) run stand	stand/groom (sta/grm) stand/nurse (sta/n	ur), and walk (additionally for fawns the behaviors
stand, ranning (starran), ran, stand,	, stand Broom (sta Brin), stand naise (sta n	ar), and want (additionally for fawing the oblighter)

A.F./ Fawn	Group	Eat	Lay	Lay/ Curl	Lay/ Slp	Lay/ Grm	Lay/ Rum	Sta/ Rum	Run	Stand	Sta/ Grm	Sta/ Nur	Walk
AF	High	2%*	27%*	N/A	N/A	7%*	4%	1%*	1%*	25%	8%*	1%	24%
AF	Moderat e	10%*	4%*	N/A	N/A	3%	2%*	1%*	1%*	34%*	13%*	0%*	32%
AF	Low	6%	19%	N/A	N/A	3%	5%	2%	5%	23%	5%	2%	30%
Fawn	High	7%	43%^	8%	2%	7%	7%	N/A	0%^	16%^	2%^	N/A	9%^
Fawn	Moderat e	12%^	20%	1%^	1%	15%^	5%	N/A	3%	21%	5%	N/A	17%
Fawn	Low	8%	24%	6%	2%	8%	5%	N/A	4%	22%	6%	N/A	15%

Lay/Curl and Lay/Slp (lay/sleep)) percentages were compared between spleen organ imidacloprid concentrations.

*percentages that are significantly (90%CI) different than the low group percentages in the spleen for adult females

^percentages that are significantly (90%CI) different than the low group percentages in the spleen for fawns

Figure 1. Average Imidacloprid levels (ng/g) in spleen tissue of 2015 and 2016 fawns (n=62) that died prematurely compared to those that survived. Imidacloprid levels differed between those that were dead compared to alive.

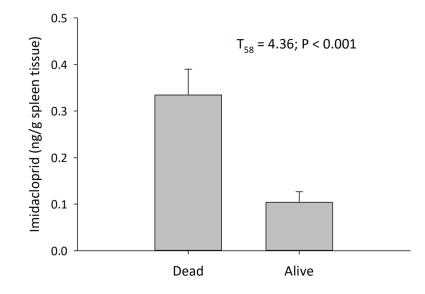


Figure 2. Linear regression between Imidacloprid levels in the spleen organs (ng/g) compared to the fawn body weight at death (kg).

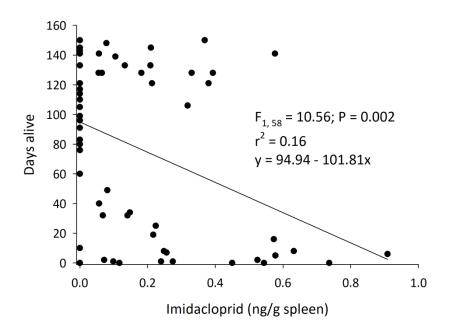
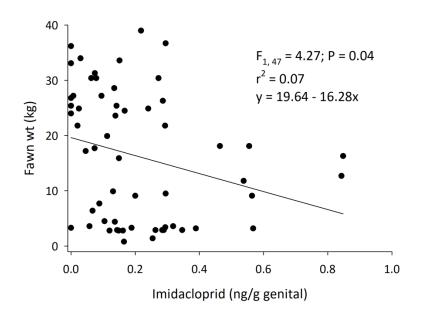


Figure 3. Linear regression between Imidacloprid levels in the genital organs (ng/g) compared to the fawn body weight at death (kg).



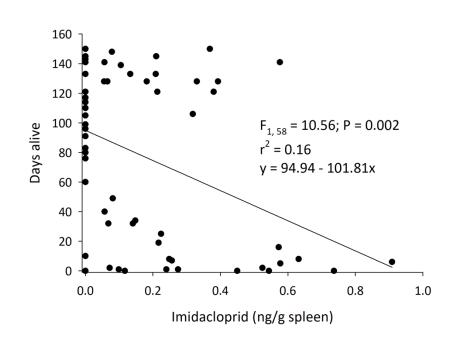


Figure 4. Linear regression between Imidacloprid levels in the spleen (ng/g) and fawn age (days)

Figure 5. Linear regression between Imidacloprid levels in the spleen (ng/g) and FT4 levels (pmol/L).

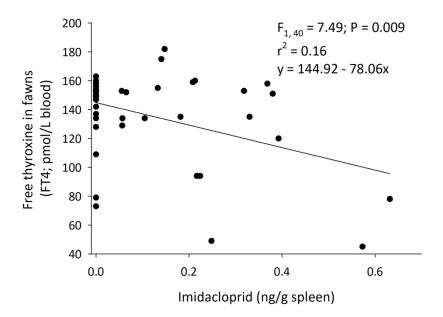


Figure 6. Linear regression between adult female genital organ weights (g) and genital Imidacloprid concentrations (ng/g).

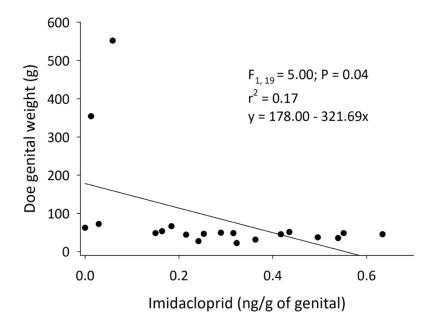


Figure 7. Fawn spleen organ weights (g) compared to Imidacloprid concentrations in spleen organs (ng/g).

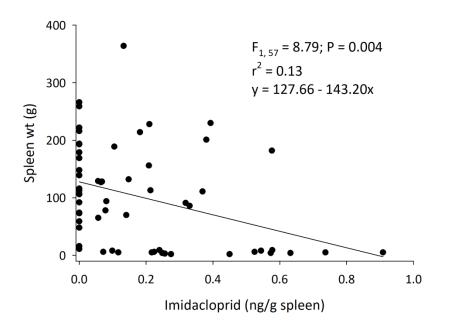


Figure 8. Fawn genital organ weights (g) compared to Imidacloprid values in the genital organs (ng/g).

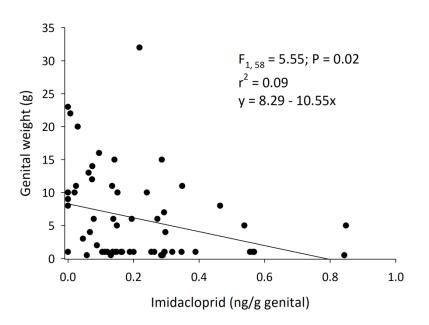
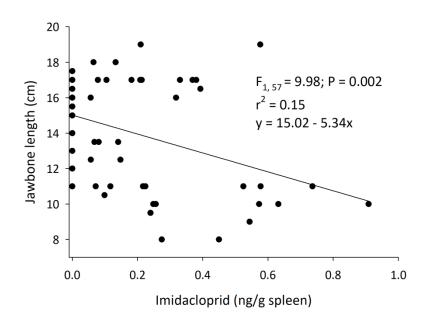


Figure 9. Linear Regression between Imidacloprid levels (ng/g) in the spleen and jawbone lengths (cm).



CHAPTER TWO: AN EVALUATION OF TECHNIQUES AND TOOLS TO MONITOR AND MODEL THE DEER POPULATION AT GREAT SWAMP NATIONAL WILDLIFE REFUGE

1.0 Abstract

Great Swamp National Wildlife Refuge (GSNWR) partnered with South Dakota State University (SDSU) and Lord Stirling Park Somerset County Environmental Education Center (SCEEC) to conduct an evaluation of techniques and tools to monitor and model the Refuge's white-tailed deer (*Odocoileus virginianus*) population. Objectives of the study were to: 1) Estimate the percentage of the population lost due to the 2014 hemorrhagic disease outbreak; 2) Obtain and compare current density estimates and other population parameters; and 3) Create a population model to predict population recovery and monitor response to future harvests. The Refuge provided SDSU Pre-hunt Spotlight Survey and harvest data, and images from a 2014 Trail-camera Survey to estimate the percentage of the deer herd lost to hemorrhagic disease. Additionally, two survey techniques (Baited Trail-camera Survey and Distance Sampling Spotlight Survey) were conducted to obtain density estimates and other population parameters for deer at GSNWR (Management and Wilderness Areas) and the adjacent Lord Stirling Park lands (SCEEC and Stables Properties). Parameters for the 10-year model (2016-2026) were obtained from historic data collected at GSNWR, the current study, and from the literature. Based on analyses, approximately 57% of the population was lost due to the disease outbreak. Also, both population survey techniques yielded comparable density estimates and all estimates for Refuge lands fell below the current management objective of 7.7 deer/km². The most recent estimate (combined Management and Wilderness Areas for the 2015 Fall/Winter post-hunt period) was 2 deer/km². Similar densities between the Management and Wilderness Areas were attributed to differences in hunting pressure between the two areas, as habitat quality in the Management Area was better suited for the species. Greater density on the adjacent SCEEC Property (12 deer/km²) likely was due to differences in annual harvest strategies between the two agencies and the timing of the survey. The Distance Sampling Spotlight Survey with the use of a FLIR device was the most suitable technique for the Management Area, but the Trail-camera Survey was more appropriate for the Wilderness Area. Population surveys, harvest data, and the 2014 Trail-camera Survey provided evidence that the Refuge experienced an influx of immigrants (predominantly males) after the disease outbreak. The population model predicted the herd would recover to pre-outbreak levels in 2017 (3 years following the outbreak), and reach a density of 7.7 deer/km² after the 2019 fawning season. The model allows for future impacts on the population to be monitored by incorporating actual numbers of deer harvested during annual hunting seasons and modifying parameter estimates, as needed.

2.0 Introduction

Great Swamp National Wildlife Refuge (GSNWR) partnered with South Dakota State University (SDSU) to conduct an evaluation of techniques and tools to monitor and model the Refuge's white-tailed deer (*Odocoileus virginianus*) population. This inventory and monitoring project provided information to aid in carrying out Objective 4.1 of GSNWR's Comprehensive Conservation Plan (CCP) and Environmental Assessment (U.S. Fish and Wildlife Service 2014a). Objective 4.1 seeks to "maintain the deer population at a level that does not negatively impact wildlife habitat and the integrity of ecological communities and provides quality, safe, compatible hunting opportunities according to State regulations and seasons through a refuge permit system." The Refuge has been conducting an annual white-tailed deer firearm hunt since 1974. Strategies to carry out Objective 4.1 include the Refuge continuing to obtain population trend data and adjusting bag limits when necessary. The deer herd is monitored from information collected on harvested animals, and from 2000 to 2014, a Pre-hunt Spotlight Survey. According to the CCP, the Refuge proposed to provide additional hunting opportunity by adding a fall archery hunt for deer, which would precede the current shotgun and muzzleloader season.

In the mid-2000s, harvest and pre-hunt spotlight survey data indicated that GSNWR's white-tailed deer density had dropped below the management objective (~7.7 deer/km²). Also, prior to the 2007 hunt, the herd experienced an outbreak of hemorrhagic disease (epizootic hemorrhagic disease; EHD), which further impacted the population. [Note: Hemorrhagic disease is a fatal disease that is caused by multiple viruses; two types of EHD virus and five types of blue-tongue virus. It is spread by a biting midge (*Culiciodes spp.*) that thrives in warm, wet conditions. Little can be done to control outbreaks and the most appropriate response is to reduce harvest in succeeding years (Hewitt 2011).] Beginning in 2007, the bag limit was changed (from an unlimited number of antlerless deer and one antlered buck per hunter, to one antlerless deer and one antlered buck per hunter, in September 2011, the herd experienced a second and more severe outbreak of EHD, which set the population

back again. This prompted further bag limit restrictions beginning in 2012 (one deer, either sex per hunter). The regulation changes of 2012 appeared to have been effective at reducing overall hunting pressure and creating conditions for population recovery. For example, although the Pre-hunt Spotlight Survey index suggested numbers were still down, the doe-to-buck ratio (2.8:1.0) indicated more does in the population in 2013, and other ancillary information obtained during the 5-day hunt (i.e., hunters reporting seeing more deer in 2013 and a greater percent hunter success occurred 2013 than the previous year) supported a growing herd. At the time, it was determined that before re-instituting more liberal regulations, there was a need to work with SDSU to conduct a more formal evaluation on the status of the population (Grant/Cooperative Agreement F14AC01179, Period of Performance 9/16/14 - 9/30/17). However, in 2014, just before the start of the study, the herd experienced a third outbreak of hemorrhagic disease (blue-tongue). This shifted the focus of the project from studying a recovering deer herd to understanding the impacts of hemorrhagic disease on the population and determining population recovery time.

Monitoring the deer herd in an adaptive management framework entails periodically reviewing current survey techniques, trying new techniques, and updating monitoring protocols when necessary. The Refuge's annual Pre-hunt Spotlight Survey had been conducted each year in August over a 4-to-5-day period to index population trends and provide information on buck age structure; information on buck age structure also was obtained from harvested animals. Although helpful, the Pre-hunt Spotlight Survey index provided only a minimum estimate of deer density (e.g., 2 deer/km² in 2013) in the 1,662-hectare Management Area, and could not be extrapolated to the 1,359hectare Wilderness Area due to markedly different management and habitat types between the two areas. Further, while the Spotlight Survey index responded to the 2011 population crash, based on a recent review on the utility of the technique (Collier et al. 2013), the index may not be sensitive enough to detect smaller, incremental changes in the population. Due to the recent population decline, as well as plans to open a fall archery hunt as outlined in the CCP (U.S. Fish and Wildlife Service 2014), it was welltimed to obtain current population parameters using multiple techniques, compare the various techniques, and incorporate parameters into a mathematical model to predict population recovery time and monitor future impacts from annual harvests.

In addition to obtaining population trend data and adjusting bag limits, another strategy outlined in the CCP under Objective 4.1 stated that the Refuge would "continue to coordinate with adjacent land managers, including county environmental education centers (EEC) and the New Jersey Department of Environmental Protection (NJ DEP) Division of Fish and Wildlife, to encourage cooperative, managed deer hunts." As a result, GSNWR reached out to the Somerset County Environmental Education Center (SCEEC) to increase collaboration in deer management efforts by incorporating adjacent Lord Stirling Park land (SCEEC and Stables Properties), about 405 hectares, into the study.

Objectives of the study were to: 1) estimate the percentage of the deer population lost due to hemorrhagic disease using an analysis of Pre-hunt Spotlight Survey and harvest data, and a refuge Trail-camera dataset 2) obtain estimates of the deer population and other population parameters at GSNWR and the adjacent Lord Stirling Park lands (SCEEC and Stables Properties) using two survey techniques (Distance Sampling Spotlight Survey and Baited Trail-camera Survey) 3) compare density estimates and evaluate other factors for the two population survey techniques 4) incorporate population parameters into a mathematical model to predict population recovery time and monitor future harvest impacts on the population.

3.0 Methods

The Refuge provided Pre-hunt Spotlight Survey and harvest data to estimate the percentage of the deer herd lost to hemorrhagic disease in 2014. Also, as part of a study to detect carnivores on the Refuge (Wagnon et al. 2016), one of the survey techniques consisted of monthly monitoring 20 trail-cameras that were placed systematically along management roads surrounding the wetland impoundments. The image dataset contained non-target animals, including white-tailed deer. This dataset also was provided for the study, to get a second, independent estimate of the percentage of the population lost due to hemorrhagic disease.

To obtain density estimates for deer at GSNWR and the adjacent Lord Stirling Park lands (SCEEC and Stables Properties) (Figure 1), we used two survey techniques: 1) Quality Deer Management Association's (QDMA) Baited Trail-camera Survey (Thomas 2010); and 2) Distance Sampling Spotlight Survey (Buckland et al. 2001; Foccardi et al. 2005). For the Distance Sampling Spotlight Survey, we followed protocols used for deer populations throughout New Jersey by NJ DEP Division of Fish and Wildlife (S. Predl, NJ DEP Division of Fish and Wildlife, Unpublished Report; U.S. Fish and Wildlife Service 2012). Prior to the hemorrhagic disease outbreak, during August 2014, a Distance Sampling Spotlight Survey for deer was completed by Refuge staff and the dataset also was provided to us for the project.

3.1 Baited Trail-camera Survey

The Baited Trail-camera Survey design followed protocols developed by QDMA (Thomas 2010). Sampling units consisted of 65-hectare (0.1 km²) square plots distributed across survey areas. Using ArcMap 10.3.1 (ArcGIS 10.3.1, Esri, Inc., Redlands, California), a grid consisting of 20 plots was overlaid onto the Management and Wilderness Areas of the Refuge (Figure 2), and 3 plots were overlaid onto the SCEEC Property (Figure 3). The center of each plot marked the location of each baited trailcamera setup. Trail-camera locations were found by entering UTM coordinates of plot centers into a GPS Unit (GARMIN GPSmap 62, Lenexa, Kansas) and hiking to the locations. Locations occasionally were adjusted, such as when the center of the sampling unit occurred in unsuitable habitat (e.g., in the middle of a wetland), for ease of access, or to take advantage of an existing deer trail. Each baited trail-camera station consisted of a motion activated Cuddeback Ambush Infrared camera (NonTypical Inc., De Pere, Wisconsin) attached to a tree or stake (about 3 feet above ground), facing a bait pile (50 pounds of corn located 10-12 feet north of the trail-camera). Sites were marked by a numbered sign behind each bait pile (Figure 4) and any obstructive vegetation between the sign and camera was cleared (Thomas 2010). Cameras were programmed to capture photos at 3-minute intervals when triggered, including infrared photos taken at night. (Note: This differed from the suggested 5-minute interval based on QDMA guidelines, as the Cuddeback Ambush IR Cameras did not have a 5-minute interval option.)

The survey was timed to occur after the annual deer hunts (November 1 and November 5-8, in 2014; and October 31, and November 4-7 in 2015), but before remaining bucks shed their antlers (Feldhamer et al. 2003) so that bucks could be uniquely identified by their antlers. The duration of each survey was 3 weeks. The first week served as a pre-baiting week to acclimate deer to the survey area, and during the second and third weeks of the survey, trail-cameras were activated to record deer activity at the baited stations. Every 2-5 days, sites were visited to check and maintain the cameras, and replace corn if necessary. At the end of each survey week, SD cards were collected from the trail cameras to analyze the images. Photos were separated into folders labeled "Bucks" (Figure 5), "Does", "Fawns" (Figure 6), "Unknowns" (Figure 7) and "Other Wildlife" (Figure 8). Bucks were then identified individually by their antlers and were further separated into a "Unique Bucks" folder (Figure 9). Totals from each of these folders were analyzed using the QDMA's Trail-camera Survey Computation Form (Figure 10). Output yielded number of does per buck, fawns per doe, acres per deer, and deer per square kilometer (Thomas 2010).

3.2 Distance Sampling Spotlight Survey

The Distance Sampling Spotlight Survey for deer followed protocols used by NJ DEP, Division of Fish and Wildlife (S. Predl, NJ DEP Division of Fish and Wildlife, Unpublished Report). According to the guidelines, surveys can be conducted any time of year, but ideally, are conducted during the Spring season (March or April), after the hunting season ends but prior to leaf-out. Additionally, surveys should be repeated at least two to three times per week on nights with favorable weather conditions (little to no fog, wind or rain) until \geq 50 independent observations of groups of deer are recorded. For

GSNWR surveys (Management Area and including the Lord Stirling Park Stables Property), each survey night, beginning one hour after sunset, a driver and 2-3 observers traveled all accessible roads (≤10 mph) in and around survey areas, spotlighting (both sides of the road) for deer (1-3 million candlepower spotlights). In addition to two, 3 million candlepower spotlights, a Forward Looking Infrared (FLIR) device also was used to detect deer. When deer were spotted, we recorded the number observed in the group, UTM location, and perpendicular distance (using a Leupold 112178/RX-1000i with DNA Digital Laser Rangefinder) from the truck to the deer (Figure 11). Information [date, location, transect length (km), transect area (km²), number observed, and perpendicular distance (km)] was incorporated into Program Distance (Thomas et al. 2010), which calculated a population density estimate with a 95% CI.

3.3 Comparison of Population Survey Techniques

Results from the two population survey techniques were compared and evaluated for future use at GSNWR. First, density estimates obtained for the Management Area from the 2014 (Pre-hunt, Pre-hemorrhagic disease outbreak) Distance Sampling Spotlight Survey and 2014 (Post-hunt) Baited Trail-camera Survey were compared after accounting for mortality from hemorrhagic disease and hunter harvest that occurred between the two survey periods. Additionally, the 2014 (Post-hunt) Trail-camera Survey estimate was directly compared to the 2015 (Pre-fawning) Spotlight Survey density estimate, as these estimates were obtained within a few months of each other, prior to significant changes in population size. Finally, the 2015 (Pre-fawning) Spotlight Survey density estimate was compared to the 2015 (Post-hunt) Trail-camera Survey estimate, after accounting for changes in the population due to various parameters (i.e., sex ratio, pregnancy rate, recruitment, fawn predation rate, and 2015 harvest). In addition to comparing density estimates, the two techniques also were evaluated based on other factors, including survey cost, number of personnel needed, survey duration, suitability for Management Area versus Wilderness Areas, and data obtained.

3.4 Population Model

A logistic growth white-tailed deer population model (Jenks et al. 2002) was created for deer at GSNWR. The purpose of the model was to determine population recovery time following the 2014 hemorrhagic disease outbreak and provide estimates of the annual harvest needed to maintain the herd at the Refuge goal of 7.7 deer/km². The model was created using Microsoft Office Excel 2010 software (Microsoft Corporation, Redmond, Washington) and run for a 10-year period (2016-2026). Parameter calculations for each year were provided so that values could be modified to predict future harvest impacts on the population and increase model accuracy.

Population parameters for the model were obtained from historic data collected at GSNWR, this study, and from the literature (Table 1). Required parameters included initial population size, proportion of adult males and females comprising the population, pregnancy rate, rate of increase for the population, fawn sex ratio, fawn survival rate, and harvest rate for males and females. The initial population size was based on the 2015 Post-hunt Baited Trail-camera density estimate for the combined Management and Wilderness Areas of the Refuge (2 deer/km², or 71 deer for the 4 km² Refuge; Table 2). Numbers of adult males and females comprising the population were determined by multiplying average proportions of bucks (0.34) and does (0.66) observed during annual Pre-hunt Spotlight Surveys conducted in the Management Area (2007-2013; U.S. Fish

and Wildlife Service 2014b) by the total number of deer (71); after 2016, numbers for both sexes were calculated by adding the total population of males and females after harvest to the number of male and female fawns that survived the previous year. The number of reproductive females was determined by multiplying the number of females comprising the population by an estimated reproductive rate of 0.85 (Table 1.; Stoll and Parker 1986). Recruitment, or the number born into the population, involved multiplying the number of reproductive females by a population rate of increase of 1.3. This value was based on an average of corpora lutea counts from harvested does at GSNWR (1971-1976 and 1982-1983; Laskowski 1984). Fawn survival rate (0.77) was obtained from a study in northcentral New Brunswick (Ballard et al. 1999). Numbers of male and female fawns that survived their first year were determined by multiplying the total number of fawns that survived by the proportions of male and female fawns born (0.5). This value was based on a sex ratio of 1:1 that was determined from fawn captures at GSNWR (1971 and 1972; Table 1; Koch 1972). Finally, average harvest rates (the proportions of the total population that were harvested) for males (0.24) and females (0.05) were obtained by averaging estimated harvest rates, using results of the 2012-2015 harvests (when bag limits were consistent at 1 deer, either sex per hunter), and density estimates from the 2012-2014 Pre-hunt Spotlight Survey and 2015 Baited Trail-camera Survey (See Appendix I for calculations). Once the deer herd reached the Refuge goal of 7.7 deer per km², the harvest rate was modified to maintain that density.

Assumptions of the model were: 1) Initial population density and other parameter estimates accurately represented the deer herd at GSNWR; 2) Deer densities in the Management and Wilderness Areas remained similar; 3) Adult mortality from causes other than hunting was negligible (adult survival rate = 1.00) and hunting mortality was additive during the 10-year period; 4) Immigration equaled emigration; 5) For Models #1 and #3, harvest rate (based on the current bag limit of 1 deer, either sex per hunter) remained constant for the 10-year period; and 6) For Models #2 and #4, harvest rate (based on the current bag limit of 1 deer, either sex per hunter) remained constant for the 10-year period; and 6) For Models #2 and #4, harvest rate (based on the current bag limit of 1 deer, either sex per hunter) remained constant until a density of 7.7 deer/km² was reached.

4.0 Results

4.1 Predicting percentage of the deer population lost to hemorrhagic disease in 2014

Estimates of the percentage of the population lost to hemorrhagic disease were obtained using two independent datasets (Refuge harvest and Pre-hunt Spotlight Survey index data, and a trail-camera image dataset). A linear regression analysis using Program SYSTAT 13.0 (Systat Software, Inc., San Jose, California) of harvest and Pre-hunt Spotlight Survey index data (2000 – 2013) indicated that the survey data correlated with total harvest ($r^2 = 0.77$; Appendix II). Based on this, using the linear regression equation [Total Harvest = 11.631 + 0.906 (Pre-hunt Spotlight Survey Index)] with a Pre-hunt Spotlight Survey index of 66 animals in 2014, the predicted mean total harvest in 2014 would have been 71 deer had the blue-tongue outbreak not occurred, or, with a 95% CI, somewhere between 53 and 90 animals. We estimated the percentage of the population lost to hemorrhagic disease to be 57% to 75%, based on the total number of actual harvested deer in 2014 (n = 23) and the lower (53) and upper (90) 95% CI values of the predicted mean total harvest.

Trail-camera images of deer at 20 survey sites in the Management Area of the Refuge (June-October 2014; Wagnon et al. 2016) were summarized using an automatic storage and analysis program (Harris et al. 2010). Images were considered independent when separated by a 60-minute time interval. The percentage of the population lost due to hemorrhagic disease was 57%, based on the total number of images of deer taken in August (n = 193, the month that the Pre-hunt Spotlight Survey was conducted) and September (n = 82, the month of the disease outbreak) of 2014 (Table 3). We speculated that the increase in number of images observed in October (n = 166) coincided with overall increases in deer movements during the fall breeding season.

4.2 Baited Trail-camera Survey

The Baited Trail-camera Survey was conducted: 1) post-hunting season in the Management Area of the Refuge, in 2014 and 2015, 2) post-hunting season in the Wilderness Area, in 2015, and 3) during the hunt on Lord Stirling Park SCEEC Property (but Post-GSNWR hunt), in 2015 (Table 2, Figure 1). Appendix III contains the total numbers of bucks, does, and fawns recorded in images at each Baited Trail-camera Survey site location at GSNWR (Management and Wilderness Areas) and the SCEEC Property. A total of 19, 65-hectare square plots were surveyed in the Management Area of GSNWR from 22 November to 14 December 2014. The QDMA computations yielded a density estimate of 2 deer/km² for the 19 plots, with 0.04 does/buck, 0.25 fawns/doe, and 144.5 acres/deer (357 hectares/deer) (Table 2; Appendix IV). [Note: At site #19, the camera malfunctioned resulting in no data being collected at that site, and at site #12, the camera was stolen. However, the survey for site #12 was repeated with a replacement camera, resulting in a different survey period (December 16-30, 2014) for this site. Bait piles at 4 sites (#4, #7, #10, and #11) became submerged in water during the last week of the survey period. Flooded sites were moved (≤ 250 ft) from the original site, and left out the equivalent number of days corn had been inaccessible to deer (1 – 2 days).]

Twenty, 65-hectares square plots (the same sites surveyed in 2014) were surveyed in the Management Area of GSNWR from 11 December 2015 to 1 January 2016 (Table 2). Results indicated 3 deer/km², 0.24 does/buck, 1.4 fawns/doe, and 97 acres/deer (240 hectares/deer). Twenty, 65-hectare square plots were surveyed in the Wilderness Area of GSNWR from 11 November to 6 December 2015. Analyses for the Wilderness Area indicated 3 deer/km², 0.21 does/buck, 0.83 fawns/doe, and 82 acres/deer (203 hectares/deer). [Note: Due to the low number of images (seven images over a 2-week period) obtained from site #14 in the Wilderness Area, a new camera was placed at the site for an extra week (1 December 2015 to 8 December 2015); however, no change in photo frequency was detected.] A combined estimate (40, 65-hectare square plots) for the Management and Wilderness Areas was 2 deer/km², 0.25 does/buck, 1.1 fawns/doe, and 105 acres/deer (259 hectares/deer). Three, 65-hectare plots were surveyed in the Lord Stirling Park SCEEC Property from 11 November to 6 December 2015. The QDMA computations yielded a density estimate of 12 deer/km², 0.42 does/buck, 1.4 fawns/doe, and 20 acres/deer (49 hectares/deer) (Table 2). [Note: The camera at site #3 malfunctioned and was left out an extra week (1 December to 8 December 2015).]

In 2014, 32% of 693 images at survey sites in the Management Area included non-target wildlife species (Table 4). In 2015, 18% of 1,350 images, 6% of 857 images, and 30% of 2,014 images were non-target wildlife species in the Management Area, Wilderness Area, and SCEEC Property, respectively. Non-target wildlife included the Common raccoon (*Procyon lotor*), Eastern wild turkey (*Meleagris gallopavo*), Other bird species [ring-necked pheasant (*Phasianus colchicus*) and songbird spp.], America black bear (*Ursus americanus*), Eastern gray squirrel (*Sciurus carolinensis*), Eastern coyote (*Canis latrans*), American red fox (*Vulpes vulpes fulvus*), and Canada goose (*Branta canadensis*) (Table 4, Figure 8).

4.3 Distance Sampling Spotlight Survey

The Distance Sampling Spotlight Survey was conducted in the Management Area of GSNWR in 2014, during the pre-hunting period (August). In 2015, during the post-2014 hunting season/pre-fawning period (March/April), the survey was conducted in Management Area of GSNWR and Lord Stirling Park Stables Property (Table 2). During the August 2014 surveys, a total of 89 groups (totaling 141 deer) were recorded from GSNWR, with group sizes ranging from 1 to 6 deer. Deer density in the Management Area in August 2014 was estimated at 5 deer/km² (95% CI, 4-7 deer/km²). During the March/April 2015 surveys, we recorded 45 groups (totaling 72 deer) from GSNWR (29 groups totaling 42 deer), and Lord Stirling Park Stables Property (16 groups totaling 30 deer), with group sizes ranging from 1 to 6 deer. Deer density in the Management Area during March/April in 2015 was estimated at 3 deer/km² (CI 95%, 2-4), and in the Lord Stirling Park Stables Property, 7 deer/km² (CI 95%, 1-8).

4.4 Comparison of Population Survey Techniques

Based on three comparisons of density estimates obtained from the Management Area using the Baited Trail-camera and Distance Sampling Spotlight Surveys, the techniques yielded similar results. As stated above, the 2014 (Pre-hunt, Pre-hemorrhagic disease outbreak) Spotlight Survey density estimate (5 deer/km²; CI 95% 4-7) was compared to the 2014 (Post-hunt and Post-hemorrhagic disease outbreak) Trail-camera Survey density estimate (2 deer/km²; Table 2). After accounting for loss of the herd due to hemorrhagic disease (57%) and harvest (n = 23), the 2014 Spotlight Survey estimate was 1 deer/km² (CI 95% 1.3 - 4.3), and the 95% confidence interval for this estimate included the Trail-camera Survey estimate. Additionally, the 2014 (Post-hunt) Trailcamera Survey estimate (2 deer/km²) was compared to the 2015 (Pre-fawning) Spotlight Survey density estimate (3 deer/km²; CI 95% 2-4). The post hunt estimate was lower than the pre-fawning estimate, falling outside of the lower end of the 95% CI. Finally, the 2015 (Pre-fawning) Spotlight Survey density estimate (3 deer/km² (CI 95% 2-4) was compared to the 2015 (Post-hunt) Trail-camera Survey estimate (3 deer/km²). After accounting for changes in the population based on various parameters [sex ratio (34%) females, 66% males), pregnancy rate (85%), recruitment (1.3), fawn predation rate (77%), and 2015 harvest (n=14)], the 2015 (Pre-fawning) Spotlight Survey estimate was 4 deer/km² (CI 95% 2-6), and the 95% CI for this estimate included the Trail-camera Survey estimate.

Although, overall, both survey techniques yielded similar density estimates, other factors that were compared, differed (Tables 5 and 6). The Distance Sampling Spotlight Survey was less expensive, required fewer people, and required less time to complete than the Baited Trail-camera Survey. Additionally, Spotlight Survey generated a density estimate that included a 95% confidence interval. However, the Spotlight Survey was not a viable technique for use in the Refuge's Wilderness Area as the survey required use of a vehicle. Thus, despite its disadvantages, the Trail-camera Survey was the more

appropriate technique for the Wilderness Area. Results of the Trail-camera Survey also provided estimates of doe-to-buck and fawn-to-doe ratios.

4.5 Population Model

Four, 10-year (2016-2026) models were created for the GSNWR deer population (Tables 7-11). Model #1 maintained consistent harvest rates associated with the current bag limit of 1 deer, either sex per hunter (Tables 7 and 8). The population reached the Refuge goal of 7.7 deer/km² after the fawning season in 2019, 5 years following the 2014 hemorrhagic disease outbreak. Continuing the current bag limit resulted in further population increases, to a density of 64 deer/km² in 2026. For Model #2, initial harvest rates were maintained until 2019, but then incrementally increased to determine the number of males and females that should be harvested to maintain a population density of 7.7 deer/km² (Table 9). Thus, harvest rates for both males and females were increased by 22% (0.46 and 0.27 for males and females respectively) in 2019, and then an additional 16% (0.62 and 0.43 for males and females, respectively) from 2020 to 2026. Model #3 incorporated the actual harvest of adult males (n = 20 and 16) and females (n = 6 and 5)obtained from 2016 and 2017 harvest records into Model #1 (Table 10). Additionally, based on actual harvest data, new average male and female harvest rates were calculated from the actual harvest numbers and population estimates in 2016 and 2017 (Male harvest rate was 0.28 and 0.18 for 2016 and 2017, respectively, average = 0.23; Female harvest rate was 0.08 and 0.08 for 2016 and 2017, average = 0.08). The new harvest rates were incorporated into the model from 2018 to 2026. Results were similar; the population reached a density of 7.7 deer/km² in 2019, and continued to increase to 51

deer/km² in 2026. For Model #4, new harvest rates were maintained until 2019, but then changed to determine the number of males and females that should be harvested to maintain a population density of 7.7 deer/km² (Table 11); harvest rates for both males and females were increased by 2% in 2019 and an additional 36% from 2020 to 2022, then reduced by 4% from 2023 to 2026.

5.0 Discussion

Over the past ten years, the GSNWR white-tailed deer herd has had three significant late-summer outbreaks of hemorrhagic disease (2007, 2011, and 2014). The type of virus that caused the 2014 disease outbreak throughout Morris County, New Jersey (including GSNWR and SCEEC lands) was a blue-tongue virus, typically found in the south (NJ DEP Division of Fish and Wildlife 2017). Deer in warm, wet regions of the country such as the southeastern United States have developed antibodies to hemorrhagic disease, which reduces impacts on these populations (Stallknecht et al. 2015). Conversely, in northern states, when drought years create favorable conditions for midge populations (i.e., during late summer/early fall when water sources become more concentrated and low water levels produce mudflat habitats), severe hemorrhagic disease outbreaks have occurred (Sleeman et al. 2009; Hewitt 2011; and Stallknecht et al. 2015). Actual percentages of populations lost to the disease are difficult to find, as pre- and postoutbreak population estimates are not available for many populations (Fischer et al. 1995, Stallknecht et al. 2002, Gaydos et al. 2004). Gaydos et al. (2004) reported that a population of deer in Harding and Hampshire Counties, West Virginia (32 km²) was reduced by 20% from a hemorrhagic disease outbreak. In Kentucky, Roughton (1975)

reported that 65% of the deer from a 2-hectare captive deer facility in Mammoth Cave National Park, died from the disease. Based on anecdotal information, the 6 km² Shiawassee National Wildlife Refuge in Saginaw, Michigan, lost about half of its deer herd when an outbreak occurred in 2012 (E. Dunton, Shiawassee National Wildlife Refuge, Saginaw, Michigan, Personal Communication). In New Jersey, deer deaths due to hemorrhagic disease were first reported in 1955. Findings from this study indicated that during the 2014 outbreak, 57% to 75% of herd was lost to the disease, although analyses of the two independent datasets indicated the percentage lost probably was closer to the low end of that range. Deer populations have been reported to recover to pre-outbreak levels in one to five years, depending on the severity of the outbreak, the extent that harvest is reduced, and habitat quality (Hewitt 2011, Bestul 2014). Based on comparing the density of adults reported in the Management Area during Pre-hunt Deer Spotlight Survey in 2014 (66 deer were observed, or a density of 4 deer/km²), to adult densities estimated by the population model (Tables 7 - 11), the GSNWR herd was predicted to recover to pre-outbreak levels in 2017, or three years following the 2014 disease outbreak.

All density estimates for Refuge lands fell below the management objective of 7.7 deer/km² and densities obtained for the Management and Wilderness Areas were similar (estimates between the two areas differed by less than 1 deer/km²). However, based on habitat requirements for the species, comparable estimates between the two areas were not expected as the Management Area supported a greater abundance of habitat that deer typically prefer (forest and woodlands interspersed with, and adjacent to, early successional and edge-type habitats; Rieucau et al. 2007; Van Moorter et al. 2009);

currently the Management Area contains about 522 hectares of early successional habitats (31% of the area) interspersed among forested and wetland impoundment habitats. Conversely, upland habitats within in the 1,359-hectare Wilderness Area are nearly 100% mature or maturing forested habitat types; U.S. Fish and Wildlife Service 2014a). Additionally, the fawn-to-doe ratio in the Management Area (1.4:1.0) was higher than that of the Wilderness Area (0.83:1.0), also supporting better habitat quality that would result in greater recruitment in the Management Area. Similar densities between the Management and Wilderness Areas likely were due to differences in hunting pressure between the two areas.

Historically, fewer deer have been harvested in the Wilderness Area compared to the Management Area. This undoubtedly is due to reduced access to this area, but also, at least in the recent past [from 2007 to Present (2017)], the fact that only muzzleloader hunters could hunt in the Wilderness Area. Muzzleloaders are single-shot, versus the multi-shot shotgun, which encourages more selective hunting, ultimately reducing hunter success (U.S. Fish and Wildlife Service 2014b). Additionally, the number of muzzleloader hunters declined after 2012, when an incentive for hunting the Wilderness Area was removed. Muzzleloader hunters had been exempted from the earn-a-buck rule in place from 2007 to 2011, when the bag limit was one antlerless and one antlered deer per hunter. The incentive was eliminated when the bag limit was reduced in 2012, to one deer, either sex per hunter (GSNWR, Unpublished Data).

Unlike deer density comparisons on GSNWR, density in the adjacent SCEEC Property (12 deer/km²) was considerably greater than that of the adjacent Management Area (3 deer/km²). Differences in densities did not appear to be due to habitat quality, as SCEEC lands also were managed for early successional habitats (U.S. Fish and Wildlife Service 2014a; J. Parks, SCEEC, Basking Ridge, New Jersey, Personal communication), and fawn-to-doe ratios were the same for the two areas. Additionally, the discrepancy in estimates could not be explained by low sample size for the SCEEC Property Survey (n =1,278-hectare plots in the SCEEC Property versus n = 20, 65-hectare plots in the Management and Wilderness Areas). Analyses of an additional random set of three sampling units on the Refuge still yielded a low estimate (2 deer/km²). Most likely, the increased density observed on the SCEEC Property was due to differences in annual harvest strategies between the two agencies and the timing of the survey.

Annual harvest strategies differed markedly between GSNWR and SCEEC. To compare, in the two years leading up to the study, GSNWR's public deer hunt took place over five days (one youth hunt day followed by four regular hunt days) within a 1-week period and throughout the entire 3,022-hectare Refuge [except in designated safety zones (537 hectares; U.S Fish and Wildlife Service 2014b) and a 24-hectare No Hunting area]. Hunters harvested deer from tree stands or on foot by shotgun or muzzle loading rifle. Hunting over bait was prohibited, and deer drives were permitted on two of the regular hunt days. In 2013, 107 hunters harvested 37 deer; and in 2014, 103 hunters harvested 23 deer (GSNWR Unpublished Data). In contrast, hunting on the SCEEC Property was carried out by Bernards Township Protective Association. The hunt took place over 17 days distributed irregularly over a two-month period and included the entire 202-hectare parcel. One to three hunters hunted per day. Baiting occurred two weeks prior to the first hunt date and continued throughout the hunting period (October 14 – December 2). Deer were harvested from tree stands or on foot, primarily by bow, although shotguns

and muzzle loading rifles also were used. In 2013, and 2014, 4 and 0 deer were harvested, respectively. When corrected for area, based on the numbers of deer harvested per km² (for GSNWR, 2 deer/km² in 2013 and 1 deer/km² in 2014; for SCEEC Property, 2 deer/km² in 2013 and 0 deer/km² in 2014), on average, the number of deer harvested at GSNWR was only slightly greater than that of the SCEEC Property (1 deer/km² versus 1 deer/km²). Probably baiting, in conjunction with limited hunting pressure on the SCEEC Property contributed to more deer congregating on the Property.

It also is possible that deer could have moved onto the SCEEC Property from the Refuge in response to the disturbance created during the regular gun season of the GSNWR annual deer hunt (64 hunters harvested 17 deer from Nov. 4 to Nov. 7, 2015), which occurred the week before the SCEEC survey began (Nov. 11, 2015). Home range sizes of deer in the northeast have been documented to range about 0.65 km² to 2.6 km² (Tierson et al. 1985; Swihart et al. 1995; and Kilpatrick et al. 2001), although significant movements (up to 29 kilometers) by 1.5-2.5-year-old males have been documented during the fall breeding season (Tierson et al. 1985). The Passaic River separates the SCEEC Property from the Management Area of GSNWR, but movement by deer between the two parcels is possible. In fact, we documented images of the same 'Unique Bucks' on both properties on two occasions during the 2015 surveys [Unique Buck #4 was documented on Plot #6 the Wilderness Area of the Refuge on November 23 and on SCEEC Plot #2 on November 27 (approximately 6 km. straight-line-distance); Unique Buck #3 was documented on Plot #10 the Wilderness Area on November 24 and on SCEEC Plot #1 on December 2 (approximately 5 km. straight-line-distance)]. Thus,

movements by deer onto SCEEC lands from the Refuge could have temporarily and artificially increased densities of deer on this property, inflating the density estimate.

Deer density in the adjacent Lord Stirling Park Stables Property (7 deer/km²) also was greater than that of the Refuge. However, the 95% confidence interval was wide (2 to 20 deer/km²), due to relatively few number of observations collected on the property (16 groups, totaling 30 deer). The difficulty in obtaining observations of deer during the 2015 surveys was due, in part, to the severe decline of the herd from the hemorrhagic disease outbreak. Additionally, in 2015, there were constraints on the dates in which the survey could be completed, and 4 of the 5 observation nights had rainy weather conditions. As mentioned earlier, ideally surveys should be repeated at least two to three times per week on nights with favorable weather conditions (little to no fog, wind or rain) until \geq 50 independent observations are obtained (S. Predl, NJ DEP Division of Fish and Wildlife, Unpublished Report). Therefore, definitive conclusions regarding deer densities at the Lord Stirling Park Stables Property could not be made.

Both population survey techniques yielded comparable density estimates indicating either technique could be used to obtain various population parameters. However, our findings differed from Roberts et al. (2006), who compared the two techniques for surveying the endangered Florida Key deer. They found that density estimates from the Spotlight Survey were lower than those of Baited Trail-camera Surveys. Different conclusions could be due to a modification to the methods that we made to the Distance Sampling Spotlight Surveys. In addition to two, 3 million candlepower spotlights to detect deer, we also used a FLIR device. Belant and Seamans (2000) tested three survey methods for detecting deer (FLIR device, spotlight, and night vision goggles) in Ohio and found that the FLIR device detected more deer than the spotlight. Likely, the FLIR device improved detectability of deer during our surveys, increasing accuracy of the Distance Sampling Spotlight Survey.

Although results of the two surveys were similar, based on comparing other factors, the Distance Sampling Spotlight Survey appeared to be the most suitable technique for the Management Area. In fact, the Spotlight Survey methods required only minor additions to the data that were collected during the Pre-hunt Deer Spotlight Surveys conducted from 2000 to 2014. Therefore, data obtained from future surveys could be used to generate estimates that are comparable to historic surveys (to aid in evaluating population trends), as well as generate a more accurate density estimate with a 95% CI. Further, while the Trail Camera Survey results included ratios of does-to-bucks, and fawns-to-does, the Distance Sampling Spotlight Survey Technique could easily be modified by conducting surveys in October (Davis 1940; Kranz 1974), to collect data that enables both the ratio of does-to-bucks and fawns-to-does to be estimated. In fact, Pre-Hunt Deer Spotlight Surveys conducted by the Refuge in the past included does-to-buck ratios. Finally, the Spotlight Survey Technique currently is used throughout the state of New Jersey by the NJ DEP Division of Fish and Wildlife to monitor deer populations and we also conducted the survey on the adjacent Lord Stirling Park Stables Property as part of this study. Results obtained from these and future GSNWR surveys could be shared with partner agencies to allow local and regional density comparisons to be made for white-tailed deer, ultimately aiding management of the species.

Despite its disadvantages, when compared to the Distance Sampling Spotlight Survey, the Baited Trail-camera Survey was the more appropriate technique for surveying the Wilderness Area, simply because it did not require the presence of a road system to conduct the survey, and the Wilderness Area is roadless. Also, because the survey could be conducted in both areas of the Refuge, direct comparisons of densities and other population parameters could be made between the Management and Wilderness Areas. Another advantage of the Trail-camera Survey technique was that, unlike the Spotlight Survey, it also provided additional population parameter estimates useful for understanding the population. For example, based on survey data collected in the Management Area in 2014 and 2015, the herd showed a clear indication of population recovery from the blue-tongue outbreak. Fawn-to-doe ratios (0.25:1.0 and 1.4:1.0 for 2014 and 2015, respectively), doe-to-buck ratios (0.04:1.0 and 0.24:1:0), and density estimates (2 deer/km² and 3 deer/km² for 2014 and 2015, respectively) all increased over the two-year period. The response of deer we observed at GSNWR was typical for a recovering population, due to the density dependent effect of fawn recruitment increasing in response to a reduced number of does having greater access to high-quality habitat with less competition (McCullough et al. 1990).

The population model provided a solid foundation for a continuing process to accurately represent the deer herd at GSNWR. Periodically, assumptions and parameter estimates should be reviewed as new and/or more accurate information becomes available, and the model should be updated accordingly. For example, Assumption #1 of the model was that initial population density and other parameter estimates accurately represented the deer herd at GSNWR. The fact that 1) our density estimates were similar using the Baited Trail-camera and Distance Sampling Spotlight Survey techniques (estimates between the two areas differed by <0.4-0.8 deer/km²), and 2) the 2015 Baited

Trail-camera Survey results were similar between the Management and Wilderness Areas, gave us confidence to generate an overall estimate for the Refuge for the initial population size. However, the buck-to-doe ratio obtained from the Baited Trail-camera survey was heavily skewed toward males. This could have been due, in part, to male dominance and sexual segregation and at bait stations (McCoy et al. 2011; Donohue et al. 2013); thus, ratios from the survey may be more useful as population trend data. As a result, we used the average proportions of bucks and does observed during annual Prehunt Spotlight Surveys (2007-2013; U.S. Fish and Wildlife Service 2014b) to calculate the initial adult male and female populations for the model; these values were skewed toward females. Although Spotlight Surveys are commonly used to monitor deer populations (Collier et al. 2007; Roberts et al. 2006; and Larue et al. 2007), in south Texas, the surveys were found to be biased toward does (Fafarman and Deyoung 1986). Thus, it is possible we overestimated the number of adult females comprising the population. However, the general trend that antlered bucks are harvested at higher rates than does or fawns due to hunter preference and the fact that bucks are less wary during the mating season (Coe et al. 1980) holds true for deer at GSNWR (GSNWR, Unpublished Data), supporting a greater density of does at GSNWR. Still, Refuge Prehunt Spotlight Surveys were conducted during the July/August time-period. Kranz (1974) reported that surveys conducted in October yielded more accurrate doe-to-buck ratios. In theory, ratios obtained from future Pre-hunt Spotlight Surveys conducted in October could be incorporated into the model to increase model accuracy. Another parameter, fawn survival rate, was obtained from a study in New Brunswick (Ballard et al. 1999). Of the fawns that died from predation, the majority were killed by coyotes and

black bears, both of which occurred on the Refuge (Wagnon et al. 2016). However, cause specific mortality information collected on fawns at GSNWR could potentially yield a more accurate estimate of fawn survival for the model.

Assumption #2 of the model was that deer densities in the Management and Wilderness Areas remained similar. As harvest strategies change, increased hunting pressure in the Wilderness Area could result in reduced densities in this Area. In response, the current model could be used as a guideline to create two separate models for the Management and Wilderness Areas.

Assumption #3 of the model was that adult mortality from causes other than hunting was negligible (adult survival rate = 1.00) and hunting mortality was additive during the 10-year period. However, additional hemorrhagic outbreaks could occur, requiring adjustments to adult and fawn survival rates. Based on our findings, under the assumption that the disease impacts both sexes and all age classes equally, mortality rates could be adjusted in the model to reflect a loss of some percentage of the population. An estimate of the percentage of the population lost could be determined from the model, based on the total number of actual deer harvested and the predicted total harvest that year. For adults, the new survival rate for the year of the outbreak should be incorporated into the predicted number of males and females harvested columns (Table 8, Columns I and J). For fawns, the new survival rate should be incorporated into the number of fawns surviving column (Table 8 Column G).

Assumption #4 of the model was that immigration equaled emigration. This probably wasn't the case for deer at GSNWR, as the population surveys, harvest data, and trail-camera survey provided evidence that the Refuge experienced an influx of

immigrants after the disease outbreak. For example, we compared the 2014 (Post-hunt) Trail-camera Survey estimate directly to the 2015 (Pre-fawning) Spotlight Survey density estimate, because these estimates were obtained within a few months of each other, prior to expected significant changes in population size. However, the post-hunt estimate was lower than the pre-fawning estimate, falling just below the lower end of the 95% CI, indicating some level of immigration may have occurred. In addition, actual numbers of males harvested during the 2016 and 2017 seasons (20 and 16 deer, respectively) were much greater than those predicted by the model (6 and 9 deer, respectively), and the number of images of deer from the trail-camera survey doubled from September (outbreak period; n = 82) to October (post-outbreak period; n = 166), providing more evidence of immigration. Thus, it is likely that the Refuge experienced some level of immigration after the 2014 disease outbreak, and that the animals were dominated by males. The significant reduction of the deer herd due to the outbreak created conditions that favored immigration onto the Refuge from surrounding areas (optimal habitat combined with little intraspecific competition; Bowyer et. al. 2014). Further, since the disease outbreak ended toward the beginning of the breeding season, influxes of individuals likely would be expected to be skewed toward males, due to increased movements by bucks during the breeding season, especially 1.5 to 2.5- year-old bucks (Ozoga and Verme 1985; Tierson et al. 1985). Having a greater number of males in the population would not necessarily affect overall population modeling results due to the polygamous breeding behavior exhibited by the species (Clutton-Brock 1989); Model #1 and Model #3 yielded comparable results. However, continued differences between

predicted and actual harvest values (outside of initial responses following disease outbreaks) should be remedied by incorporating immigration into the model.

6.0 Management Implications

This study provided the first density estimates for deer at GSNWR, since 1974, when helicopter surveys were conducted to document population size (GSNWR, Unpublished Data). Not only did our findings determine the severity of the impact from the 2014 hemorrhagic disease outbreak on the population, but also, density estimates indicated the herd was well below the stated management goal of 7.7 deer/km². Additionally, based on two independent survey techniques, we obtained an initial population size for creating a population model. The 10-year model predicted: 1) population recovery time from the disease outbreak (3 years); 2) the year that the density would reach 7.7 deer/km² (2019); and 3) the number of deer that would need to be harvested each year to maintain the management goal (Tables 9 and 11). The model also is adaptive, in that it allows future impacts on the population to be monitored by incorporating actual numbers of deer harvested during annual hunts, and modifying parameter estimates when needed (Table 11). The SCEEC could use the GSNWR model as a basis to create a deer population model for their lands.

The Distance Sampling Spotlight Survey with the use of the FLIR device should be conducted periodically in the Management Area to verify population model parameters. Unlike past Pre-hunt Spotlight Surveys, the survey should be carried out in October to obtain more accurate doe-to-buck and fawn-to-doe ratio estimates. Baited Trail-camera surveys also should be repeated, as needed, in the Management and Wilderness Areas to determine if densities remain similar (an assumption of the population model), especially if harvest strategies increase hunting pressure in the Wilderness Area. During the years that surveys are conducted, GSNWR could continue their partnership with SCEEC, combining resources to survey the three properties (GSNWR, and Lord Stirling Park SCEEC and Stables Properties).

According to the population model, under the current bag limit and with no additional disease outbreaks, the herd reached a density of 7.7 deer/km² after the 2019 fawning season. Thus, to keep the deer herd from growing past that level, the harvest rate would need to increase by 2019 (Table 11). This could be achieved, initially, by increasing the bag limit to 1 antlerless and 1 antlered deer per hunter, and reinstating the earn-a-buck requirement for shotgun hunters, and/or initiating a fall archery season. Subsequently, each year, actual harvest of adult males and females could be incorporated into Model #3 (Table 11, Columns I and J) to predict future population response. Harvest rates, strategies, and bag limits could be adjusted as needed.

Objective 4.1 of the CCP seeks to maintain the deer population at a level that 1) does not negatively impact wildlife habitat and the integrity of ecological communities and 2) provides hunters a quality, safe, and compatible deer hunt (U.S. Fish and Wildlife Service 2014a). However, based on forest health evaluations conducted at GSNWR in 2008 and again, in 2015, Van Clef (2015) suggested that a reduced herd [~4 deer/km² (M. Van Clef, Ecological Solutions, LLC, Great Meadows, NJ, Personal Communication; Hopewell Valley Deer Management Task Force 2010)] still could offer a high-quality hunting experience by providing large bucks to hunters through a Deer Management Program that emphasized the harvest of does. Based on the forest health evaluations, improvements to native understory from 2008 to 2015 were attributed to reduced browsing by deer from a combination of harvest management and the recent outbreaks of hemorrhagic disease (Waller and Alverson 1997; Kilpatrick et al. 2001; Van Clef 2015). In fact, native understory cover on the Refuge more than tripled that of New Jersey's statewide average. However, measurements still hadn't reached levels considered to be a healthy forest (e.g., native understory cover of >70%, non-native understory cover <10% and seedling browse rates of <10%). Van Clef (2015) recommended that GSNWR gauge the success of its Deer Management Program through the attainment of healthy forests. Based on our findings, to maintain a density of approximately 4 deer/km² would require an increase in the number of deer harvested by the 2018 hunting season.

7.0 Acknowledgments

Funding for this study was provided by U.S. Fish and Wildlife Service GSNWR and South Dakota Agricultural Experiment Station. Special thanks to GSNWR Deputy Refuge Managers S. Henry and L. McLaughlin for providing staff support for the project, and S. Herdman and A. ^{Mi}tchell, for their assistance with the population surveys. We also thank GSNWR staff (M. McMenamin, J. Rosenburg, J. Pimentel, D. Miller, and G. Molnar), Lord Stirling Park Staff (M. Margentino and J. Parks), and Friends of GSNWR Volunteers (J. Bell, D. Sharpe, D. Bertram, J. Balwierczak, G. Annibal, R. Dufort, J. Mulvey, J. Schmidt, S. Gruber, K. Ward, D. Young, C. Woodwards, K. Woodwards, C. Schroeder, and L. Gould) for help with the surveys. Historic data collected on deer by B. Koch, D. Spencer, H. Laskowski, and C. Bitler, was extremely helpful for creating the population model. Thanks to C. Wagnon of Frostburg State University for providing the 2014 trail-camera white-tailed deer image dataset and K. Cudmore, for assistance with Program Distance. Finally, we wish to thank E. Michel, L. McLaughlin, J. Parks, and M. Van Clef for reviewing portions of this report.

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Fish and Wildlife Service Great Swamp National Wildlife Refuge, Basking Ridge, NJ. 166 pp.

Waller, D. and W. Alverson 1997. The white-tailed deer: a keystone herbivore. Wildlife Society Bulletin 25: 217-226 Table 1. Parameter estimates used to create a 10-year population model (2016-2026) for white-tailed deer at Great Swamp National

Parameter	Source
Initial Population Size	2015 Post-hunt Baited Trail-camera Survey results for GSNWR (combined Management and Wilderness Areas).
Proportion of Bucks	Average proportion of bucks observed during annual Pre-hunt Spotlight Surveys conducted in the Management Area (2007-2013; U.S. Fish and Wildlife Service 2014)
Proportion of Does	Average proportion of does observed during annual Pre-hunt Spotlight Surveys conducted in the Management Area (2007-2013) U.S. Fish and Wildlife Service 2014)
Reproductive Rate	Stoll, R. and W. Parker. 1986. Reproductive performance and condition of white-tailed deer in Ohio, The Ohio Journal of Science 86 (4):164-168.
Rate of Increase	Average of corpora lutea counts from harvested does at GSNWR (1971-1976 and 1982-1983; Laskowski 1984).
Fawn Survival Rate	Ballard, W., H. A. Whitlaw, S. J. Young, R. A. Jenkins, and G. J. Forbes. 1999. Predation and survival of white-tailed deer fawns in northcentral New Brunswick. <u>The Journal of Wildlife Management</u> 63:574-579.

Wildlife Refuge (GSNWR).

Table 1 continued.

Fawn Sex Ratio	Sex ratio of fawns captured at GSNWR (1971 and 1972; Koch
	1972).
Harvest Rate of Bucks	Average of the estimated harvest rate of bucks (2012-2015), when
	bag limits were consistent at 1 deer, either sex per hunter, based on
	GSNWR harvest data (2012-2015), and density estimates, using the
	2012-2014 Pre-hunt Spotlight Survey data and 2015 Baited Trail-
	camera Survey; See Appendix I for calculations.
Harvest Rate of Does	Average of the estimated harvest rate of does (2012-2015), when
	bag limits were consistent at 1 deer, either sex per hunter, based on
	GSNWR harvest data (2012-2015), and density estimates, using the
	2012-2014 Pre-hunt Spotlight Survey data and 2015 Baited Trail-
	camera Survey; See Appendix I for calculations.

Table 2. Density estimates and population parameters for white-tailed deer at Great Swamp National Wildlife Refuge [GSNWR; Management Area (MA) and Wilderness Areas (WA)] and adjacent Lord Stirling Park lands [Somerset County Environmental Education Center (SCEEC) and Lord Stirling Stables (LSS)] using two techniques (Baited Trail-camera Survey and Distance Sampling Spotlight Survey); 2014, 2015. Trail-Camera Surveys took place after the annual hunts for GSNWR (Hunt dates for 2014 and 2015 were Nov. 4, Nov. 5-8 and Oct. 31, Nov. 4-7, respectively), and during the hunt for SCEEC Property (Hunt dates for 2015 were Oct. 28, Nov. 4-6, Nov. 18, Dec. 2 and Dec. 30; hunt dates for 2016 were Jan. 6, 20, and 27).

Location	Time Period	Survey Type	Dates	Total Hectare	No. Plots Surveyed	Deer/km	Doe:Buc k	Fawn:Do e	Hectares/ Deer
		• •		S		(95%CI)			
MA	2014: Pre-hunt	Spotlight	8/11,	1,662		5			
		Survey	8/13			(4-6)			
			8/14,						
			8/19						
MA	2014: Post-hunt	Trail-	11/22-	1,230	19	2	0.04:1.0	0.25:1.0	357
		camera	12/30						
		Survey							
MA	2015: Pre-	Spotlight	3/25,	1,662		3			
	fawning	Survey	3/26			(2-4)			
	(Post 2014 hunt)		3/31, 4/1						
LSS	2015: Pre-	Spotlight	3/30, 4/1	182		7			
	fawning	Survey				(2-20)			
	(Post 2014 hunt)								

Table 2 Contin	ued.								
MA	2015: Post-hunt	Trail-camera Survey	12/11/15 - 1/1/16	1,294	20	3	0.24:1.0	1.4:1.0	240
WA	2015: Post-hunt	Trail-camera Survey	11/11- 12/8	3,200	20	3	0.21:1.0	0.83:1.0	203
SCEEC	2015: Post-hunt	Trail-camera Survey	11/11- 12/8	480	3	12	0.42:1.0	1.4:1.0	49
MA & WA	2015: Post-hunt	Trail-camera Survey	11/11/15 - 1/1/16	6,400	40	2	0.25:1.0	1.1:1.0	259

Table 3. Independent trail-camera images of white-tailed deer at 20 survey sites in the Management Area of Great Swamp National Wildlife Refuge (June-October 2014). Images were considered independent when separated by a 60-minute time interval. Data was summarized using an automatic storage and analysis program created by Harris et al. (2010).

Camera Set Type	No. Cameras	No. Independent Images					
		June	July	Aug.	Sept.	Oct.	Total
Non-baited	20	202	229	193	82	166	872

Table 4. Non-target wildlife species detected in the Management area (MA) and Wilderness Area (WA) of Great Swamp National Wildlife Refuge and the Lord Stirling Somerset County Environmental Education Center (SCEEC) Property (2014, 2015).

Species	% of Photos in MA 2014 (n = 693)	% of Photos in MA 2015 (n = 1,350)	% of Photos in WA 2015 (n = 1,857)	% of Photos in SCEEC 2015 (n = 2,014)	
American Black Bear (Ursus americanus)	6%	0%	2%	0%	
Eastern Coyote (<i>Canis latrans</i>)	<1%	0%	0%	0%	
American Red Fox (Vulpes vulpes fulvus)	<1%	0%	0%	0%	
Common Raccoon (Procyon lotor)	4%	7%	1%	18%	
Eastern Gray Squirrel (Sciurus carolinensis)	<1%	<1%	2%	<1%	
Other bird spp. (songbirds (<i>Passeri</i>), ring-neck pheasant (<i>Phasianus</i> colchicus))	1%	3%	<1%	9%	
Canada Goose (Branta canadensis)	0%	0%	<1%	0%	
Eastern Wild Turkey (<i>Meleagris</i> gallopavo)	19%	8%	0%	2%	

Table 5. A comparison of two population survey techniques (Distance SamplingSpotlight Survey and Baited Trail-camera Survey) conducted for white-tailed deer atGreat Swamp National Wildlife Refuge (2014, 2015).

Factors	Spotlight Survey	Trail-camera Survey
Equipment Cost	\$2,002	\$5,508
Number of Personnel	4 people/ night	2-4 people/every 3-4 days
Survey Duration	1 week	3-4 weeks
Requires System of Roads	Yes	No
Data Obtained	Density Estimate	Density Estimate (Deer/km ²), Does per Buck, Fawns per Doe, Acres/Deer
95%		
Confidence Interval	Yes	No

Table 6. A comparison of costs for two population survey techniques (DistanceSampling Spotlight Survey and Baited Trail-camera Survey) conducted for white-taileddeer at Great Swamp National Wildlife Refuge, 2014 and 2015.

В	aited Trail-c	amera Sur	vey	Distan	ce Sampling	Spotlight	Survey
Item	Quantity	Price	Total	Item	Quantity	Price	Total
Ambush cuddeback IR trail camera-5 MP	20	\$99	\$1,980	Brinkman Qbeam 800-2380- W Max Million III Rechargea ble Spotlight Offroad FLIR	2	\$55	\$110
Mounting Strap	20	\$2	\$40	Scout II Thermal Night Vision Monocula	1	\$1,399	\$1,399
Deer Feed Whole Corn 50lb bag	60	\$9	\$540	r Garmin GPSMAP 64 2.6" Handheld GPS Leupold	1	\$212	\$212
Duracell Batteries	11	\$19	\$209	112178/R X-1000i with DNA Digital Laser Rangefind er	1	\$279	\$279
CuddeSafe	20	\$19	\$380	•			
Trapper Bags ZHPUAT	2	\$89	\$178				
Memory Cards Carrying Case SanDisk 8	1	\$6	\$6				
GB Secure Digital High Capacity Memory Card	40	\$7	\$280				
Master Lock Padlock	20	\$6	\$120				

Table 6 continued.

Garmin GPSMAP 64 2.6" Handheld GPS	1	\$212.99	\$212.99	
ArcGIS online Total	1	\$1,500.0 0	\$1,500.0 0 \$5,508	\$2,002

Table 7. Population Model (Model #1) for white-tailed deer at Great Swamp National Wildlife Refuge (2016 – 2026), under the current harvest management (1 deer, either sex per hunter). Parameters were initial population size (71), proportion of adult males and females comprising the population (0.34 and 0.66, respectively), pregnancy rate (0.85), population rate of increase (1.3), fawn survival rate (0.77), proportion of male and female fawns (0.50), male and female harvest rates (0.24 and 0.05, respectively). Beginning in 2017, adult male and female populations were determined by adding, from the previous year, the male and female population after harvest to the number of male and female fawns that survived. A '*'indicates the Refuge goal of 7.7 deer/km² was reached or exceeded.

		А	В	С	D	Е	F	G	Н	Ι	J	К	L	М
#1	Year	Total Pop.	Deer/km ²	Adult Male Pop.	Adult Fem. Pop.	No. Pregnant Fem.	No. Born	No. Fawns surviving	No. Male & No. Fem. Fawns	Predicted No. Males Harvested	Predicted No. Fem. Harvested	Male Pop. After Harvest	Fem. Pop. After Harvest	Tot. Pop. After Harvest
1	2016	71	2	24	47	40	52	40	20	6	2	18	45	63
2	2017	103	3	38	64	55	71	55	27	9	3	29	61	90
3	2018	145	5	57	89	75	98	75	38	14	4	43	84	127
4	2019*	203	7	81	122	104	135	104	52	19	6	61	116	177
5	2020*	281	9	113	168	143	185	143	71	27	8	86	159	245

Tabl	le 7	continued.

6	2021*	388	13	157	231	196	255	196	98	38	12	120	219	339
7	2022*	535	18	218	317	270	351	270	135	52	16	165	301	467
8	2023*	737	42	300	436	371	482	371	186	72	22	228	415	643
9	2024*	1014	34	414	600	510	663	511	255	99	30	315	570	885
10	2025*	1396	46	570	826	702	912	702	351	137	41	433	784	1217
11	2026*	1920	64	784	1135	965	1255	966	483	188	57	596	1079	1675

Table 8. Calculations for Population Model #1 for white-tailed deer at Great Swamp National Wildlife Refuge (2016 – 2026), under the current harvest management (1 deer, either sex per hunter). Parameters were initial population size (71), proportion of adult males and females comprising the population (0.34 and 0.66, respectively), pregnancy rate (0.85), population rate of increase (1.3), fawn survival rate (0.77), proportion of male and female fawns (0.50), male and female harvest rates (0.24 and 0.05, respectively). Beginning in 2017, adult male and female populations were determined by adding, from the previous year, the male and female population after harvest to the number of male and female fawns that survived.

# Yr	A Tot. Pop.	B Deer Per km ²	C Adult Male Pop.	D Adult Female Pop.	E No. Pregnant Females	F No. Born	G No. Fawns Surviving	H No. Male & No. Female Fawns	I Predicted No. Males Harveste d	J Predicted No. Females Harveste d	K Male Pop. After Harvest	L Female Pop. After Harvest	M Total Pop. After Harvest
1 2016	71	2	71×0.34= 24 A1×0.34	71×0.66=47 A1×0.66	47×0.85=40 D1×0.85	40×1.3= 52 E1×1.3	52×0.77=40 F1×0.77	40×0.5=2 0 G1×0.5	24×0.24=6 C1×0.24	47×0.05=2 D1×0.05	24–6=18 C1-I1	47–7=45 D1-J1	18+45=63 K1+L1
2 2017 3	63+40=103 M1+(2×H1) 90+54=145	3	18+20=38 K1+H1 29+27=57	45+20=64 L1+H1 61+27=89	65×0.85=55 D2×0.85 89×0.85=75	50×1.3=71 E2×1.3 75×1.3=98	71×0.77=55 F2×0.77 98×0.77=75	55×0.5=2 7 G2×0.5 75×0.5=3	38×0.24=9 C2×0.24 57×0.24=1	64×0.05=3 D2×0.05 89×0.05=4	38–9=29 C2-I2 57–14=43	64–3=61 D2-J2 89–4=84	29+61=90 K2+L2 43+84=127
2018 4 2019	M2+(2×H2) 127+76=20 3 M3+(2×H3)	15	K2+H2 43+38=81 K3+H3	L2+H2 84+38=122 L3+H3	D3×0.85 122×0.85=1 04 D4×0.85	E3×1.3 104×1.3=1 35 E4×1.3	F3×0.77 135×0.77=1 04 F4×0.77	$8 \text{ G3} \times 0.5$ $104 \times 0.5 =$ 52 $\text{G4} \times 0.5$	4 C3×0.24 81×0.24=1 9 C4×0.24	D3×0.05 122×0.05= 6 D4×0.05	C3-I3 81–19=61 C4-I4	D3-J3 122–6=116 D4-J4	K3+L3 61+116=177 K4+L4

Table	8	continued.
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1 4010	o comunaca.	•											
5 202 0	177+104=2 81 M4+(2×H4)	9	61+52 =113 K4+H4	116+52=16 8 L4+H4	168×0.85=1 43 D5×0.85	143×1.3=1 85 E5×1.3	185×0.77=1 43 F5×0.77	143×0.5= 71 G5×0.5	113×0.24= 27C5×0.24	168×0.05= 8 D5×0.05	113- 27=86 C5- I5	168-8=160 D5-J5	86+160=245 K5+L5
6 202 1	245+142=3 88 M5+(2×H5)	13	86+71=15 7 K5+H5	160+71=23 1 L5+H5	231×0.85=1 96 D6×0.85	196×1.3=2 55 E6×1.3	255×0.77=1 96 F6×0.77	196×0.5= 98 G6×0.5	157×0.24= 38C6×0.24	231×0.05= 12 D6×0.05	157– 38=120 C6-I6	231- 12=219 D6-J6	120+219=33 9 K6+L6
7 202 2 8	339+196=5 35 M6+(2×H6)	18	120+98=2 18 K6+H6	219+98=31 7 L6+H6	317×0.85=2 70 D7×0.85	270×1.3=3 51E7×1.3	351×0.77=2 70 F7×0.77	$270 \times 0.5 =$ 135 $G7 \times 0.5$	$218 \times 0.24 =$ 52 C7 \times 0.24	317×0.05= 16D7×0.05	218- 52=165 C7-I7	317- 16=301 D7-J7	165+301=46 7 K7+L7
202 3	467+270=737 M7+(2×H7)	24	165+135=3 00 K7+H7	301+135=4 36 L7+H7	436×0.85=3 71 D8×0.85	371×1.3=4 82 E8×1.3	482×0.77=3 71 F8×0.77	371×0.5= 186 G8×0.5	206×0.24= 72 C8×0.24	436×0.05= 22 D8×0.05	300- 72=228 C8-I8	436- 22=415 D8-J8	228+415=64 3 K8+L8
9 202 4	643+372=101 4 M8+(2×H8)	58	228+186=4 14 K8+H8	415+186=6 00 L8+H8	600×0.85=5 10 D9×0.85	510×1.3=6 63 E9×1.3	663×0.77=5 11 F9×0.77	511×0.5= 255 G9×0.5	414×0.24= 99 C9×0.24	600×0.05= 30 D9×0.05	414- 99=315 C9-I9	600- 30=570 D9-J9	315+570=88 5 K9+L9
10 202 5	885+510=139 6 M9+(2×H9)	10 4	315+255=5 70 K9+H9	570+255=8 26 L9+H9	826×0.85=7 02 D10×0.85	702×1.3=9 12 E10×1.3	912×0.77=7 02 F10×0.77	702×0.5= 351G10× 0.5	570×0.24= 137 C10×0.24	826×0.05= 41 D10×0.05	570- 137=433 C10-I10	826- 41=784 D10-J10	433+784=12 17 K10+L10
11 202 6	1217+702=19 19 M10+(2×H10)	64	433+351=7 84K10+H1 0	784+351=1 135L10+H1 0	1135×0.85= 965 D11×0.85	965×1.3=1 255 E11×1.3	1255×0.77= 966 F11×0.77	966×0.5= 483 G11×0.5	784×0.24= 188 C11×0.24	1135×0.05 =57 D11×0.05	784- 188=596 C11-I11	1135- 57=1079 D11-J11	596+1079=1 675K11+L1 1

Table 9. Population Model (Model #2) for white-tailed deer at Great Swamp National Wildlife Refuge (GSNWR; 2016 - 2026), under the current harvest management (1 deer, either sex per hunter), until the Refuge goal of 7.7 deer/km² was reached (2019*). At that time, harvest rates for both males and females were increased by 22% in 2019 and then an additional 16% from 2020 to 2026 to maintain the desired density.

		А	В	С	D	Е	F	G	Н	Ι	J	К	L	М
#2	Year	Total Pop.	Deer/km ²	Adult Male Pop.	Adult Fem. Pop.	No. Pregnant Fem.	No. Born	No. Fawns surviving	No. Male & No. Fem. Fawns	No. Males Harvested	No. Fem. Harvested	Male Pop. After Harvest	Fem. Pop. After Harvest	Tot. Pop. After Harvest
1	2016	71	2	24	47	40	52	40	20	6P	2P	18	45	63
2	2017	103	3	38	64	55	71	55	27	9P	3P	29	61	90
3	2018	145	5	57	89	75	98	75	38	14P	4P	43	84	127
4	2019*	203	7	81	122	104	135	104	52	37R	33R	44	89	133
5	2020	236	7.7	95	141	120	156	120	60	59R	61R	36	80	117
6	2021	236	7.7	96	140	119	155	119	60	60R	60R	37	80	116
7	2022	236	7.7	96	140	119	154	119	59	60R	60R	37	80	116
8	2023	235	7.7	96	139	118	154	118	59	59R	60R	36	79	116
9	2024	234	7.7	96	138	118	153	118	59	59R	59R	36	79	115
10	2025	233	7.7	95	138	117	152	117	59	59R	59R	36	78	115
11	2026	232	7.7	95	137	116	151	117	58	59R	59R	36	78	114

P = Predicted number of males and females that would be harvested based on an average harvest rate determined from GSNWR harvest data. R = Recommended number of males and females that should be harvested based on increasing the harvest rate to maintain the deer herd at 20 deer/km² [0.46 and 0.27 for males and females, respectively (an increase of 22%) in 2019, and 0.62 and 0.43 for males and females respectively (an additional increase of 16%) from 2020 to 2026].

Table 10. Population Model (Model #3) for white-tailed deer at Great Swamp National Wildlife Refuge (GSNWR;2016 – 2026), under the current harvest management (1 deer, either sex per hunter) and incorporating actual numbers of harvested adult males (20 and 16) and females (6 and 5) from the 2016 and 2017 annual harvests.

		А	В	С	D	Е	F	G	Н	Ι	J	K	L	М
#3	Year	Tota 1 Pop.	Deer/k m ²	Adult Male Pop.	Adul t Fem. Pop.	No. Pregna nt Fem.	No. Born	No. Fawns survivi ng	No. Male & No. Fem. Fawns	No. Males Harveste d	No. Fem. Harvest ed	Male Pop. After Harve st	Fem. Pop. After Harve st	Tot Pop. After Harve st
1	2016	71	2	24	47	40	52	40	20	20A	6A	4	41	45
2	2017	85	3	24	61	52	67	52	26	16A	5A	8	56	64
3	2018	116	4	34	82	69	90	69	35	8P	7P	26	75	101
4	2019	171	6	61	110	93	121	93	47	14P	9P	47	101	148
5	2020	241	8	94	148	126	163	126	63	22P	12P	72	136	208
6	2021	334	11	135	199	169	220	169	85	31P	16P	104	183	287
7	2022	456	15	189	268	227	296	228	114	43P	21P	145	246	391
8	2023	619	20	259	360	306	398	306	153	60P	29P	199	331	531
9	2024	837	28	353	484	412	535	412	206	81P	39P	271	446	717
10	2025	1129	37	478	652	554	720	554	277	110P	52P	368	600	967
11	2026	1522	51	645	877	745	969	746	373	148P	70P	497	807	1303

A = Actual number of adult males and females harvested during the annual deer hunt at GSNWR.

P = Predicted number of males and females that would be harvested based on an average harvest rate determined from GSNWR harvest data.

Table 11. Population Model (Model #3) for white-tailed deer at Great Swamp National Wildlife Refuge (GSNWR; 2016 – 2026), under the current harvest management (1 deer, either sex per hunter), until the Refuge goal of 7.7 deer/km² was reached (2019). To maintain the desired density, harvest rates for both males and females were increased by 2 % in 2019 and an additional 36% from 2020 to 2022, then reduced by 4% from 2023 to 2026.

		А	В	С	D	Е	F	G	Н	Ι	J	K	L	М
#4	Year	Tot al Pop	Deer /km ²	Adult Male Pop.	Adul t Fem. Pop.	No. Pregna nt Fem.	No. Born	No. Fawns survivin g	No. Male & No. Fem. Fawns	No. Males Harv.	No. Fem. Harv.	Male Pop. After Harvest	Fem. Pop. After Harve st	Tot. Pop. After Harve st
1	2016	71	2	24	47	40	52	40	20	20A	6A	4	41	45
2	2017	85	3	24	61	52	67	52	26	16A	5A	8	56	64
3	2018	116	4	34	82	69	90	69	35	8P	7P	26	75	101
4	2019	171	6	61	110	93	121	93	47	15P	11P	46	99	145
5	2020	238	7.7	92	146	124	161	124	62	56R	67R	36	79	115
6	2021	239	7.7	98	141	119	155	120	60	60R	65R	38	76	114
7	2022	234	7.7	98	136	115	150	115	58	60R	62R	38	73	112
8	2023	227	7.7	96	131	111	145	111	56	55R	55R	41	76	117
9	2024	229	7.7	97	132	112	146	112	56	55R	55R	42	76	118
10	2025	230	7.7	98	132	113	146	113	56	56R	56R	42	77	119
11	2026	232	7.7	98	133	113	147	113	57	56R	56R	42	77	120

A = Actual number of adult males and females harvested during the annual deer hunt at GSNWR.

P = Predicted number of males and females that would be harvested based on an average harvest rate determined from GSNWR harvest data. R = Recommended number of males and females that should be harvested based on increasing the harvest rate to maintain the deer herd at 7.7 deer/km² [0.25 and 0.10 for males and females, respectively (an increase of 2%) in 2019, 0.61 and 0.46 for males and females respectively (an increase of 36%) from 2020 to 2022, and 0.57 and 0.42 for males and females, respectively (a decrease of 4%)]. Figure 1. Study Area for the white-tailed deer population surveys: Management and Wilderness Areas of Great Swamp National Wildlife Refuge, Lord Stirling Park Somerset Country Environmental Education Center and Lord Stirling Park Stables Properties.

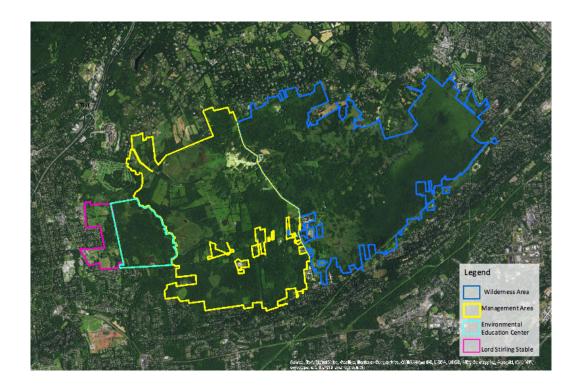


Figure 2. Sampling units [65-hectare (0.1 km²) square plots] and numbered locations of baited trail-camera stations for the Baited Trail-camera Survey in the Management Area and Wilderness Area of Great Swamp National Wildlife Refuge. The center of each unit marked the location of each baited trail-camera station.

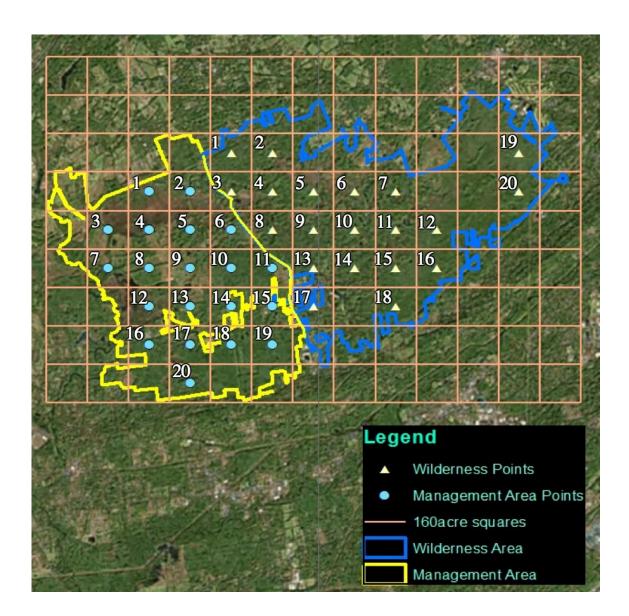


Figure 3. Sampling units [65-hectare (0.1 km²) square plots] and numbered locations of baited trail-camera stations for the Baited Trail-camera Survey in the Lord Stirling Park Somerset County Environmental Education Center Property. The center of each unit marked the location of each baited trail-camera station.

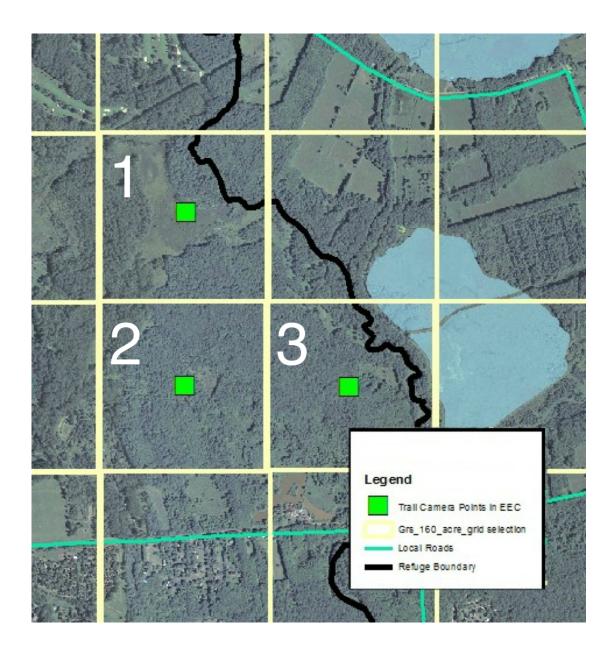


Figure 4. Baited trail-camera station in the Management Area of Great Swamp National Wildlife Refuge. Each baited trail-camera station consisted of a motion activated Cuddeback Ambush Infrared camera attached to a tree or stake (about 3 feet above ground), and facing a bait pile (50 pounds of corn located 10-12 feet north of the trail-camera). Sites were marked by a numbered sign behind each bait pile.



Figure 5. Image of two bucks at site #17 in the Management Area of Great Swamp National Wildlife Refuge, in 2014. This photo was placed into the "Buck" folder. Each buck image counted towards the total number of bucks observed as well as the number of unique bucks.



Figure 6. Photograph of a doe and fawn at site #1 in the Management Area of Great Swamp National Wildlife Refuge, in 2015. This image was placed in both the "Fawn" and "Doe" folders, and counted towards total numbers of does and fawns observed.



Figure 7. Photograph of an unknown deer at site #1 in the Management Area of Great Swamp National Wildlife Refuge, in 2015. Because there was no way to uniquely identify the deer in this image (i.e. buck, fawn, doe), the photograph was placed into an "Unknown" folder.



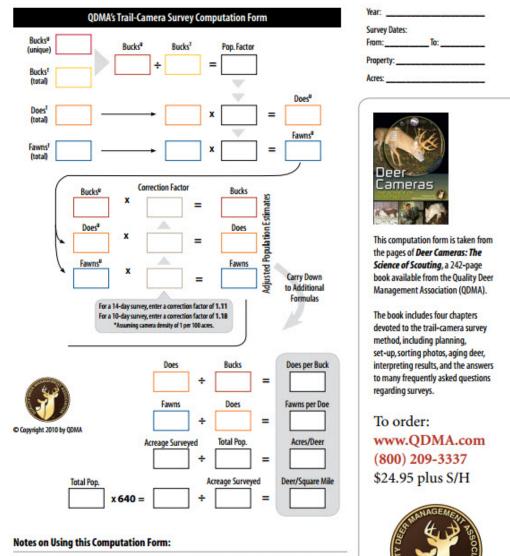
Figure 8. Image of an American black bear at a baited trail camera station (site #2) in the Management Area of Great Swamp National Wildlife Refuge. Many non-target wildlife species were observed in the images, including black bear, coyote, red fox, raccoon, gray squirrels, wild turkey, and various songbird species.



Figure 9. These three images of the same buck were taken on different days in the Wilderness Area of Great Swamp National Wildlife Refuge, in 2015. These photos were placed into the "Unique Buck" folder and counted towards the total number of unique bucks.



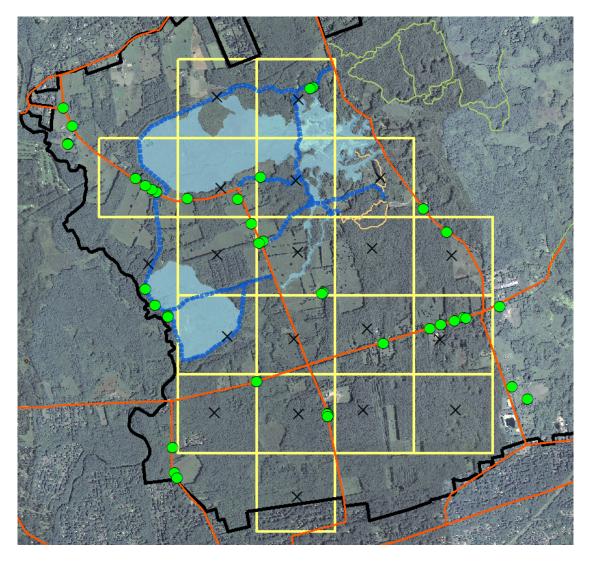
Figure 10. Quality Deer Management Association's (QDMA) Trail-Camera Survey Computation Form, where totals from all folders (i.e. "Unique Bucks", "Bucks", "Does", and "Fawns") were recorded and analyzed.



Total Deer: In sorting photos from a 14-day survey, count the total number of antlered bucks, total number of does, and total number of fawns (deer under 1 year of age). "Total" includes known repeats, so an individual deer photographed 10 times in one visit would count 10 times toward the "total" number. Unique Bucks: This is the number of unique, individual bucks that appear in your total set of photos from the 14-day survey period. For example, you may have a total of 1,000 photos of bucks, and this number includes 30 unique bucks photographed multiple times each. Unidentified: Remember to be conservative in your sorting. If you cannot confidently identify a deer as a buck, doe or fawn, do not include it in the "total" numbers for your survey.

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Figure 11. Locations (green dots) where deer or groups of deer were recording during the 2015 Distance Sampling Spotlight Survey in the Management Area of Great Swamp National Wildlife Refuge.



Appendix I. Calculations used to determine the harvest rate parameters for bucks and does at Great Swamp National Wildlife Refuge (GSNWR) to incorporate into a population model for the species. Harvest rates were estimated by averaging estimated harvest rates of bucks and does from 2012 to 2015, when bag limits were consistent at 1 deer, either sex per hunter. Calculations were based on GSNWR harvest data (2012-2015) and density estimates from the

Year	Estimated Pre-hunt Population Size of the Management Area ^a	Estimated Pre-hunt Population Size of the Refuge	No. Bucks Harves ted	No. Does Harves ted	Propor tion of Bucks Harves ted	Propor tion of Does Harves ted
2012	28 (Pre-hunt)	28 × 1.82 ^b = 50.96	13	2	0.25	0.04
2013	35 (Pre-hunt)	$35 \times 1.82^{b} = 63.7$	29	6	0.45	0.09
2014	83.4 (Pre- hunt)	$83 \times 1.82^{b} =$ 151.06	15	4	0.10	0.03
2015 [°]	48.5 (Pre- hunt)	71.2 + 17 = 88.2	13	4	0.15	0.04
					AVE: 0.24 SD: 0.15 SE: 0.08	AVE: 0.05 SD: 0.03 SE: 0.01

2012-2014 Pre-hunt Spotlight Surveys and 2015 Baited Trail-camera Survey.

^a Values were obtained from the results of the Pre-hunt Spotlight Surveys conducted at GSNWR.

^b 1.82 was the value that the total number of adults observed in the Management Area during Pre-hunt Spotlight Surveys in 2012-2014 had to be multiplied by, to calculate an overall estimate for the Refuge (Management and Wilderness Areas combined). The value was based on the 2015 density estimates, and obtained by dividing the pre-hunt population size estimate for the Refuge (88.2) by the pre-hunt estimate for the Management Area (48.5).

^c In 2015, the Baited Trail-camera Survey population size estimate for the Refuge (combined Management and Wilderness Area for the Fall/Winter post-hunt period) was 71.2 deer. Since this was a post-hunt estimate, the pre-hunt estimate (88.2) was determined by adding the total number of deer harvested during the 2015 deer hunt

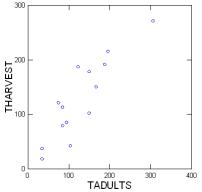
(n=17) to the pre-hunt estimate. Then, since densities between the Management and Wilderness Areas were similar, using the pre-hunt estimate (88.2 deer) for the 3,022-hectare Refuge, the pre-hunt estimate for the 1,662-hectare Management Area (48.5 deer) was determined through cross multiplication.

Appendix II. Data and output from a linear regression analysis using Program SYSTAT 13.0 (Systat Software, Inc., San Jose, California) of Great Swamp National Wildlife Refuge harvest (THARVEST) and spotlight survey index (TADULTS) data (2000 - 2013).

TADULTS	YEAR	THARVEST
196	2000	215
188	2001	191
306	2002	271
150	2003	178
123	2004	187
167	2005	150
150	2006	102
95	2007	85
85	2008	79
85	2009	113
75	2010	121
104	2011	42
35	2012	18
35	2013	37

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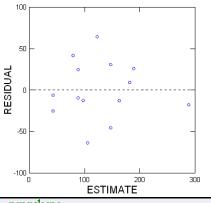
Successfully saved file P:\Wildlife\Deer\DeerData\PopulationAnalysis.syz Processed 4 Variables and 14 Cases.



▼OLS Regression

Dependent Variable	THARVEST
Ν	14
Multiple R	0.884
Squared Multiple R	0.781
Adjusted Squared Multiple R	0.763
Standard Error of Estimate	36.108

Plot of Residuals vs. Predicted Values



•	Regression Coefficients B = (X'X) 'X'Y											
Effect	Coefficient	Standard Error	Std.	Tolerance	t	p-Value						
			Coefficient			-						
CONSTANT	11.631	20.210	0.000		0.575	0.576						
TADULTS	0.906	0.139	0.884	1.000	6.541	0.000						

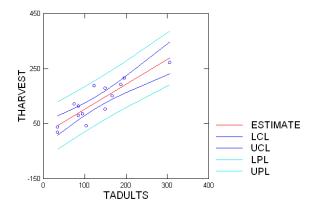
Analysis of Variance									
Source	SS	df	Mean Squares	F-Ratio	p-Value				
Regression	55,783.307	1	55,783.307	42.787	0.000				
Residual	15,645.050	12	1,303.754						

Durbin-Watson D-Statistic	
First Order Autocorrelation	0.117

Information Criteria							
AIC	143.994						
AIC (Corrected)	146.394						
Schwarz's BIC	145.911						

Г

Confidence Interval and Prediction Interval



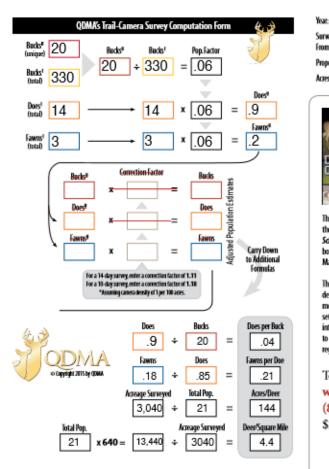
Appendix III. Total numbers of bucks, does, and fawns recorded in images at each Baited Trail-camera survey site location at Great Swamp National Wildlife Refuge Management Area (MA) and Wilderness Area (WA) as well as Lord Stirling Somerset County Environmental Education Center (SCEEC) Property (2014, 2015).

A	Yea	Site	I a4:4 J.	Long!t J	No.	No.	No.
Area	r	No.	Latitude	Longitude	Bucks	Does	Fawns
MA	2014	1	40.722088	-74.49828	0	0	0
MA	2014	2	40.722193	-74.50812	18	0	0
MA	2014	3	40.714859	-74.48911	0	0	0
MA	2014	4	40.714909	-74.49864	7	0	0
MA	2014	5	40.71376	-74.51647	4	0	0
MA	2014	6	40.714991	-74.51771	3	0	0
MA	2014	7	40.707582	-74.47965	7	0	0
MA	2014	8	40.708401	-74.48942	1	0	0
MA	2014	9	40.708053	-74.49840	31	0	0
MA	2014	10	40.707717	-74.50823	38	0	2
MA	2014	11	40.707031	-74.51650	30	0	0
MA	2014	12	40.699932	-74.48124	0	0	0
MA	2014	13	40.700981	-74.49012	0	0	0
MA	2014	14	40.700065	-74.49897	4	1	0
MA	2014	15	40.700325	-74.50703	0	0	0
MA	2014	16	40.693411	-74.47947	0	0	0
MA	2014	17	40.693506	-74.49066	106	0	1

MA	2014	18	40.693189	-74.49844	35	0	0
MA	2014	19	40.69335	-74.50863	0	4	0
MA	2014	20	40.685426	-74.49904	3	4	1
MA	2015	1	40.722088	-74.49828	55	17	13
MA	2015	2	40.722193	-74.50812	2	0	0
MA	2015	3	40.714859	-74.48911	2	0	0
MA	2015	4	40.714909	-74.49864	62	2	0
MA	2015	5	40.71376	-74.51647	12	4	0
MA	2015	6	40.714991	-74.51771	22	0	0
MA	2015	7	40.707582	-74.47965	4	0	0
MA	2015	8	40.708401	-74.48942	2	0	0
MA	2015	9	40.708053	-74.49840	4	2	0
MA	2015	10	40.707717	-74.50823	79	2	8
MA	2015	11	40.707031	-74.51650	101	2	1
MA	2015	12	40.699932	-74.48124	8	1	0
MA	2015	13	40.700981	-74.49012	4	3	0
MA	2015	14	40.700065	-74.49897	14	17	0
MA	2015	15	40.700325	-74.50703	6	1	2
MA	2015	16	40.693411	-74.47947	8	12	21
MA	2015	17	40.693506	-74.49066	4	2	6
MA	2015	18	40.693189	-74.49844	30	6	10
MA	2015	19	40.69335	-74.50863	40	34	37
MA	2015	20	40.685426	-74.49904	5	3	1

WA	2015	1	40.72932	-74.48926	19	0	6
WA	2015	2	40.72947	-74.47937	121	21	21
WA	2015	3	40.72229	-74.4893	35	0	0
WA	2015	4	40.72217	-74.47971	25	10	4
WA	2015	5	40.72213	-74.47022	15	30	3
WA	2015	6	40.72209	-74.46054	30	5	0
WA	2015	7	40.72204	-74.45114	0	0	0
WA	2015	8	40.71488	-74.47958	33	0	0
WA	2015	9	40.71491	-74.47018	21	0	0
WA	2015	10	40.71486	-74.4606	1	0	0
WA	2015	11	40.71482	-74.45101	0	0	0
WA	2015	12	40.71477	-74.44162	44	7	4
WA	2015	13	40.70769	-74.47033	2		0
WA	2015	14	40.70764	-74.46066	0	0	0
WA	2015	15	40.7076	-74.45117	0		0
WA	2015	16	40.70748	-74.44168	2	1	0
WA	2015	17	40.70046	-74.4702	3	0	0
WA	2015	19	40.70038	-74.45122	1	0	0
WA	2015	19	40.72912	-74.42241	42	4	7
WA	2015	20	40.72183	-74.42267	1	2	0
SCEEC	2015	1	40.708428	-74.52532	180	77	116
SCEEC	2015	2	40.700427	-74.52506	85	0	0
SCEEC	2015	3	40.698996	-74.51674	6	0	6

Appendix IV. Quality Deer Management Association's (QDMA) Baited Trail-camera Survey computation forms used to quantify does per buck, fawns per doe, acres per deer, and deer per square kilometer based on data collected during the surveys. Surveys were conducted at Great Swamp National Wildlife Refuge, in Management Area in 2014 and 2015; Wilderness Area in 2015, and at the adjacent Lord Stirling Somerset County Environmental Education Center Property, in 2015.



Notes on Using this Computation Form:

Total Deer: In sorting photos from a 14-day survey, count the total number of antiered bucks, total number of does, and total number of fawns (ideer under 1 year of age). "Total" includes known repeats, so an individual deer photographed 10 times to new visit would count 10 times toward the "Total" number. Unique Bucks: This is the number of unique, individual bucks that appear in your total set of photos from the 14-day survey period. For example, you may have a total of as 1,000 photos of bucks, and this number includes 30 unique bucks motod pathed multiple times each.

Unidentified: Remember to be conservative in your sorting. If you cannot confidently identify a deer as a buck, doe or fawn, do not indude it in the "total" numbers for your survey. 2014

Survey Dates: From: <u>11/22/14</u>. To: <u>12/30/14</u> Property: Management Area: GSNWR Arres: <u>3,040</u>



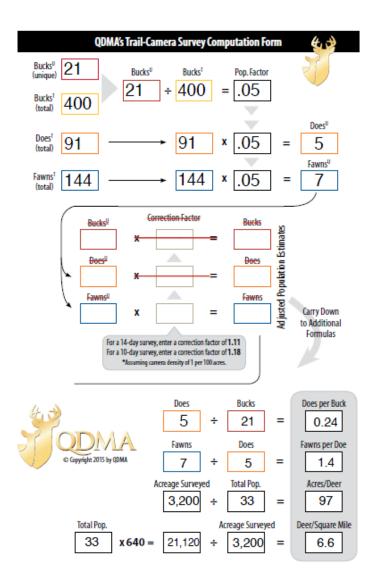
This computation form is taken from the pages of *Deer Comeros: The Science of Scouting*, a 242-page book available from the Quality Deer Management Association (QDMA).

The book includes four chapters devoted to the trail-camera survey method, including planning, set-up, sorting photos, aging deer, interpreting results, and the answers to many frequently asked questions regarding surveys.

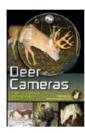
To order: www.QDMA.com (800) 209-3337 \$24.95 plus S/H



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Total Deer: In sorting photos from a 14-day survey, count the total number of antlered bucks, total number of does, and total number of fawns (deer under 1 year of age). "Total" includes known repeats, so an individual deer photographed 10 times in one visit would count 10 times toward the "total" number. Unique Bucks: This is the number of unique, individual bucks that appear in your total set of photos from the 14-day survey period. For example, you may have a total of 1,000 photos of bucks, and this number includes 30 unique bucks photographed multiple times each. Unidentified: Remember to be conservative in your sorting. If you cannot confidently identify a deer as a buck, doe or fawn, do not include it in the "total" numbers for your survey. Year: <u>2015</u> Survey Dates: From: <u>12/11/15</u> To: <u>1/1/16</u> Property: <u>Management Area</u> GSNWR Acres: <u>3,200</u>



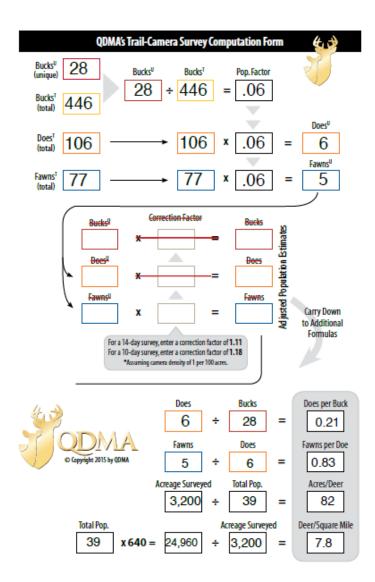
This computation form is taken from the pages of *Deer Cameras: The Science of Scouting*, a 242-page book available from the Quality Deer Management Association (QDMA).

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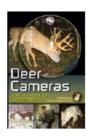
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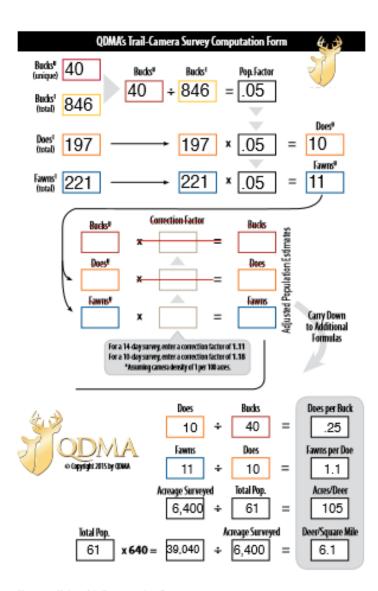
This computation form is taken from the pages of *Deer Cameras: The Science of Scouting*, a 242-page book available from the Quality Deer Management Association (QDMA).

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Unidentified: Remember to be conservative in your sorting. If you cannot confidently identify a deer as a buck, doe or fawn, do not include it in the "total" numbers for your survey.

Year:	2015/2016
Survey D	ates:
From: 1	1/8/15_lα_1/3/16
Property: GSNWR	
	6,400



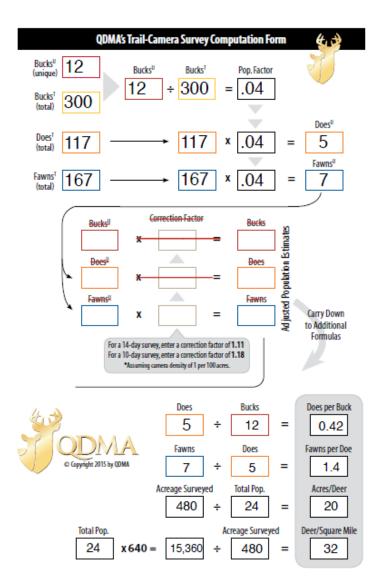
This computation form is taken from the pages of *Deer Comeras: The Science of Sconting*, a 242-page book available from the Quality Deer Management Association (QDMA).

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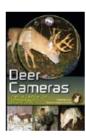


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Year:2015	
Survey Dates:	
From: 11/11/15 To: 12/8/15	
Property: Environmental Education Center	
Acres: 480	



This computation form is taken from the pages of *Deer Cameras: The Science of Scouting*, a 242-page book available from the Quality Deer Management Association (QDMA).

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