POPULATION DENSITY AND PREVALENCE OF RABIES VIRUS-NEUTRALIZING ANTIBODIES IN A NORTHERN OHIO RACCOON POPULATION

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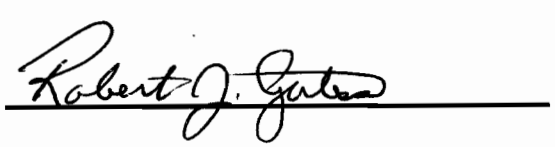

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ABSTRACT

The current oral rabies vaccination (ORV) programs across the eastern USA were established to prevent the westward spread of raccoon (*Procyon lotor*) rabies. The programs distribute vaccine baits at a density of 75 baits/km^2. However, few studies have examined the relationship of bait density and population density to sero-prevalence of rabies virus-neutralizing antibodies (RVNA). I conducted experimental baitings in August 2003 and 2004, 150 km west of the ORV zone (Sandusky, Ohio) where there was no history of raccoon rabies. I collected blood samples from live-trapped raccoons to determine sero-prevalence of RVNA, and teeth to determine prevalence of tetracycline (biomarker in bait). During April-October (2003 and 2004), I evaluated 3 mark-recapture-based estimates of raccoon population density, as well as a line-transect-based method on the 22-km^2 U. S. National Aeronautics and Space Administration Plum Brook Station in Erie County, Ohio (USA; 41° 27' N, 82° 42' W). During 2003, 41% of pre-bait serum samples were RVNA positive ($\geq$ 0.05 IU/ml), but none had titers $\geq$ 0.25 IU/ml. During the pre-2004 bait drop period (March-August) 21% of samples collected were RVNA positive and 9% had titers $\geq$ 0.25 IU/ml. After the 2003 and 2004 bait drops (September-October) only 4% of serum samples collected had high titers. Prevalence of tetracycline in post-bait teeth indicated that 17% and 27% of the population ingested baits in 2003 and 2004, respectively. I first calculated annual

minimum number known alive (MNKA) density estimates, approximating the protocol used by the U. S. Department of Agriculture Wildlife Services, and estimated an adult population size of 660 raccoons and 594 raccoons during 2003 and 2004, respectively. I also estimated size of the adult population using the catch per unit effort (CPUE) method, which yielded 438 ± 182 raccoons and 527 ± 208 raccoons for 2003 and 2004 respectively. Using program CAPTURE and model M_{bh} (heterogeneity and trap response), I estimated a population size of 619 ± 83 during 2003 and 765 ± 92 during 2004. Using Distance (version 4.1) and the line-transect data, I estimated 198 raccoons and 220 raccoons for 2003 and 2004, respectively. During 2003 and 2004 both, surveys resulted in density estimates less than the number of unique individuals captured. I note that lack of replication in the MNKA model precludes error estimates. Also, assumptions of equal probability of capture for both the MNKA and CPUE estimates were violated, likely biasing my estimates low. However, the upper limit of the CPUE estimate in both years was similar to mean estimates from the mark-recapture model. I suggest that mark-recapture would serve well in providing density information in ORV planning. Further, in situations where trapping would be difficult due to trap exposures (e.g., urban settings), estimates based on line-transect data from FLIR could provide a baseline to estimate the target population density. I attribute the low proportions of high RVNA titers and tetracycline to the high density of raccoons on the study area. I estimated an adult population of 619 ± 83 (95% CI) raccoons, using 2003 data and model M_{bh}. Assuming an annual birthrate of 1.5 juveniles per adult, 1,548 raccoons were present at the time of the 2003 baiting, so just under 1 bait was distributed per raccoon, well below the program target of 5 baits/raccoon. A high proportion of RVNA positive raccoons in

an area with no history of raccoon rabies or vaccination efforts exemplifies the need for pre-bait serology in order to accurately measure the effect of ORV distribution. I contend that without incorporating pre-bait serology and population density estimates, an ORV program could under-bait high-density populations and overestimate the number of vaccinated animals.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

The rabies viruses are members of the genus whose root word, *Lyssa*, is Greek for madness (Steele and Fernandez 1991). This describes the severe aggression and irrational behavior associated with the disease. Rabies is a zoonotic virus that, if contracted, causes encephalitis and eventual death (Rupprecht et al. 1995). The virus is spread via contact with an infected animal's saliva, through a bite or contact with a mucous membrane. Rabies is a disease of great concern, both today and throughout history, because of its ability to infect many host species including humans. From Democritus, who first described rabies in 500 B. C., to the current cases of human rabies, mostly in developing countries, the danger to humans will continue without effectively implemented control programs. There were about 50 human rabies cases per year before canine rabies was controlled in the USA in the 1950s (Fishbein and Robinson 1993). Since then reported human rabies cases contracted in the United States dropped to only 8 between 1980 and 1993. The proliferation of wildlife rabies, especially with raccoons (*Procyon lotor*) becoming the reservoir with the most documented cases in the USA (>85%) has increased concerns about human health (Dobson 2000).

With the mid-Atlantic epizootic of raccoon rabies in the 1980s, large-scale control efforts were enacted. One approach, oral rabies vaccination (ORV) programs have been ongoing across the eastern USA since the early 1990s and in Ohio since 1997. The ORV programs now include cooperation and funding from state (Ohio Departments of Health [ODH] and Natural Resources [ODNR]) and federal agencies (U. S. Department of Agriculture and Centers for Disease Control and Prevention). Between $230 million and $1 billion per year is spent on rabies prevention and post-exposure treatment in the USA alone (Fishbein and Robinson 1993). Rabies control efforts in Ohio have successfully established and maintained a barrier to prevent the westward spread of raccoon-strain rabies into Ohio and the Midwest. The barrier is currently maintained by baiting the eastern edge of Ohio once every year around August. Continued success of this vaccine barrier depends on maintaining a relatively high level of herd immunity in the target population. Combining knowledge about bait distribution densities and frequencies with the knowledge of seasonal raccoon population densities and movements will not only allow for maintenance of the current barrier but could also help the eventual eastward movement of the barrier.

The Ohio Department of Health (ODH 2002) determined the prevalence of rabies virus-neutralizing antibodies (RVNA) in raccoons after oral rabies vaccine (ORV) baits were distributed at 75, 150, and 300 baits/km^2. Sero-prevalence did not differ between the 75 (22%) and 150 (27%) baits/km^2 but there was an increase (19%) in RVNA at 300 baits/km^2. These baiting densities were evaluated without an estimate of the population density. The lack of an experimental design incorporating a population

estimate could have produced an overestimate of sero-prevalence. An important lingering question is: how does density of the target population and density of ORV baits distributed affect sero-prevalence of RNVA?

Information about the length of the period of sero-prevalence of the RVNA after uptake of ORV is important to assessing the "strength" of the vaccine barrier and determining how long the population remains protected between bait distributions. Factors like population turnover via mortality, recruitment, emigration, and immigration would directly affect the proportion of immune animals. But estimating these effects is contingent upon detecting immune animals over time. Underestimating the size of the immune class would lead to an inflation of program costs by increasing bait distribution density and frequency.

GOALS AND OBJECTIVES

The goal of this study was to measure the change in sero-prevalence of RVNA in a raccoon population of a known density, after distribution of ORV baits at a target density of 75 baits/km^2. My objectives were to 1) determine background levels of RVNA for the population, 2) estimate population densities from 3 trapping-based methods and from data obtained during line-transect surveys, 3) simulate an operational ORV distribution, and 4) determine RVNA sero-prevalence after the ORV distribution. I predicted that RVNA would not be present in the population before I distributed baits and that the level of sero-prevalence in the population would increase after the distribution of ORV baits.

THESIS FORMAT

In Chapter 2, I evaluate 4 techniques used to estimate the population density of raccoons on the National Aeronautics and Space Administrations (NASA) Plum Brook Station (hereafter referred to as Plum Brook). I evaluate the methods based on preservation or violation of assumptions along with comparisons of logistical strengths and weaknesses. This chapter is formatted for publication in the *Wildlife Society Bulletin.*

In Chapter 3, I examine the change in sero-prevalence of RVNA in the Plum Brook raccoon population before and after the distribution of ORV baits. My analysis also includes comparison of bait density, bait and vaccine ingestion and population density to sero-prevalence of RVNA. Chapter 3 is formatted for publication in the *Journal of Wildlife Management.*

BACKGROUND

RACCOON NATURAL HISTORY AND ECOLOGY

Raccoons are highly adaptable mammals with a geographic range that spans southern Canada, the Atlantic to the Pacific coasts of the USA, and south through Panama (Canadian Wildlife Services 1989). Raccoons occupy a wide range of environments from large urban cities to swamps. Hoffman and Gottschang (1977) found a mean home range size of 0.051 km^2 in a suburban population of raccoons in Ohio, while raccoon home ranges in open habitats have been reported as large as 3 km^2 (Pedlar et al. 1997). Home range size is determined mostly by habitat and resource availability. Raccoons are generally nocturnal and highly omnivorous. Their normal diet

includes fruits, berries, nuts and grains, along with crustaceans, shellfish, fish, amphibians, insects, eggs, mollusks, rodents, and young birds (Lotze and Anderson 1979). Raccoons are notorious for foraging through refuse in areas inhabited by people.

Raccoons can range in color from a dark grayish-brown to reddish-brown and even blonde. The most characteristic marking of raccoons is their "bandit" mask, an area of black-brown surrounding the eyes bordered on both sides by areas of white. Raccoons are also identified by their tail that has five to ten conspicuous brown-black rings that alternate with lighter hairs. Raccoons have small rounded ears and dark brown eyes. Their hunched posture while walking also is an identifiable raccoon trait (Lotze and Anderson 1979). Raccoons have a mean total body length around 80 cm and body weight averages 4 to 8 kg with males being generally larger than females. The estimated life span of a wild raccoon is 3-5 years with the total population being replaced over 7 years (Canadian Wildlife Service 1989).

The raccoon breeding season, in most of their range, begins during February or March, and the gestation period is approximately 63 days. Most litters of young raccoons, or kits, are born in April or May (Lotze and Anderson 1979). The female, or sow, typically raises one litter each year with a mean litter size of 3-5 kits. The female becomes solitary after mating and does not tolerate males, while the male, or boar, continues searching for mates. Female raccoons can breed in their first year, but it is highly unlikely that males will breed before their second year. Kits open their eyes around the second or third week and tooth eruption starts shortly thereafter. Kits are weaned around 2-4 months after birth and leave the den after about 8 weeks post-birth. Raccoons do not hibernate, but the kits den with their mothers during winter and become

much less active. Some young disperse during the fall, but most stay with their mother until spring. Females tend to remain in their natal territory while males usually disperse from their mother's home range, which decreases chances of inbreeding but also increases risk of predation on young males.

Raccoons have many predators including wild and domestic canids, wild felines and great horned owls (*Bubo virginianus*). The major predator of raccoons is man, taking 2-4 million pelts per year (Canadian Wildlife Service 1989). Humans also account for thousands of raccoon vehicular deaths each year. Disease is another important mortality factor for raccoon populations. Canine distemper kills thousands of raccoons annually. Rabies is another disease that is prevalent in raccoons. Rabies is especially important to humans because it is a zoonotic virus that infects many species including humans (Lotze and Anderson 1979).

RABIES ECOLOGY

One of the biggest problems affecting programs that attempt to control the spread of rabies is that the disease can be transmitted to multiple species, including humans (Fishbein and Robinson 1993). The rabies virus has many strains in the USA that include canine, skunk, bat and raccoon (Carey and McLean 1983). Because these strains can infect many different host species, the species beginning an epizootic may not be of the same species as the one experiencing the epizootic.

The initial outbreak of an epizootic can have as many as three times the number of reported infected individuals as the next and in general, the second outbreak has about 25% more cases than the third (Childs et al. 2000). The interepidemic period starts

around 45 months between first and second epidemics and shortens by an average of 5.3 months per subsequent epidemic. This is most likely due to the reduction in population density.

Rabies is spread by direct contact with an infected individual and is therefore a density-dependent disease. This is affected seasonally by many factors including movements, distribution, and behavior (Rupprecht and Smith 1994). Population reduction is one way to help reduce the likelihood of an epizootic (Broadfoot et al. 2001).

RABIES IMMUNOLOGY

The rabies virus is in the family Rhabdoviridae, genus *Lyssavirus*, a group of antigenically and genetically related, morphologically similar, negative-stranded RNA viruses (Rupprecht et al. 1995). Most exposures to rabies occur from bites by rabid animals, but any exposure of mucous membranes or open wounds to infected saliva can produce infection. The virus usually replicates in skeletal muscle cells near the inoculation site after exposure, or it can immediately attach to the peripheral nervous system (Fishbein and Robinson 1993). The virus then migrates to and attacks the central nervous system, via the peripheral nervous system, causing encephalitis; eventually spreading centrifugally throughout the peripheral nervous system and to the salivary glands were it is released into the saliva. Once in saliva the disease is easily spread to other hosts. Rabies-related encephalitis eventually causes coma followed by death. Rabies is invariably fatal after signs of disease develop.

The rabies virus antigen (located on the protein coat) is confronted by lymphocytes possessing different antibody specificities when the rabies virus enters the body (Roitt and Rabson 2000). The rabies virus antigen is recognized by specific

lymphocytes. Once the antigen is bound to the corresponding lymphocyte recognition site, the lymphocyte enlarges, proliferates and develops into antibody-forming plasma cells. The antibodies secreted by plasma cells are the same as that from the original lymphocyte. Serum antibodies then bind to the antigen of the virus and neutralize the virus. Once the antibodies are produced, they remain in the body at elevated levels. This concept is the basis for development of vaccines to combat diseases.

In the case of the vaccinia-rabies glycoprotein (V-RG) recombinant virus vaccine, the gene coding for the rabies virus glycoprotein is put into the vaccinia virus. The V-RG recombinant replicating in the raccoon, results in the rabies virus glycoprotein production that the raccoon immune system recognizes as foreign, which in turn stimulates the immune response to the rabies virus glycoprotein. The immune system then produces RVNA and the individual is protected against rabies in the event of exposure. The genetically engineered V-RG vaccine cannot cause rabies.

RABIES IN NORTH AMERICA

The rabies virus probably existed in North America before European settlement. A possible pathway could have been the Bering Strait over which the first humans entered America around 50,000 years ago (Rupprecht et al. 1995). This is supported by spoken histories from native people of the Pacific Northwest describing a sickness with symptoms similar to rabies. The first terrestrial rabies cases in the USA were reported in what is now California in 1703 (species not identified). Canine rabies outbreaks in the mid-Atlantic were probably worsened by the introduction of dogs and red foxes (*Vulpes vulpes*) used for fox hunting in the late 1700s. Canine rabies was the main focus of early rabies control programs in the USA. The program included intensive vaccination of pets

and enforcement of leash laws. Canine rabies was diagnosed more frequently than rabies among wildlife until 1960. Skunks (*Mephistis mephistis*) became the primary reservoir of rabies in 1960. Raccoons supplanted skunks as the major animal reservoir of rabies in 1989. The rabies virus can infect a number of host species including skunks, raccoons, foxes, coyotes (*Canis latrans*) and several bat species. The zoonotic aspect of rabies makes it difficult to control because targeting one species does not limit the spread of the virus in other species, nor does it protect the target species from interspecific infection.

The raccoon strain of rabies was first identified in Florida in the 1940s and remained isolated in Florida and Georgia prior to the 1970s (Rupprecht and Smith 1994). Infected raccoons from Georgia were introduced into Virginia in the mid-1970s, which was the origin of the Atlantic coast epizootic. Raccoons became the predominant wild terrestrial animal reservoir for the rabies virus by 1989. By the end of the 20th century, the disease spread northeast at a rate of 30 to 50 km/year (Childs et al. 2000). The high rate of spread is probably related to the ability of raccoons to thrive in areas of human habitation (Rupprecht and Smith 1994). Today, raccoon rabies has spread across the entire eastern seaboard and as far inland as Ohio and Alabama. Raccoon rabies also crossed into Ontario where they had their first reported case in the summer of 1999 (Rosatte et al. 2001).

RABIES IN OHIO

The first reported raccoon rabies case in Ohio was confirmed in May 1996 in Mahoning County (ODH 2002). More cases of rabid raccoons began surfacing in northeastern Ohio in 1997. The Ohio Department of Health began distributing ORV baits around the outbreak area in May 1997. After the vaccine barrier was established, raccoon

rabies cases in Ohio have dropped from 59 in 1997 to only a single case on the Pennsylvania border in 2001. Over 4 million ORV baits were distributed in 2002 alone, over an area including Ohio, Pennsylvania, Virginia, Tennessee and West Virginia. More information on the densities of raccoon populations, ORV bait uptake and sero-conversion rates is still needed (Hanlon et al. 1999).

COMBATING RABIES

Carey and McLean (1983) studied raccoon rabies in Florida before raccoons became the main rabies reservoir in the late 1980s. They determined RVNA prevalence in both epizootic and post-epizootic areas and found an average of 16.4% and 20.2% sero-prevalence of RVNA, respectively. Raccoons in enzootic areas had on average a 7.2% lower antibody prevalence than in epizootic areas, but a higher than average antibody prevalence (2.6%) than found in raccoon populations in other parts of the country. These findings suggest that there will be a naturally occurring immune class of individuals after an outbreak; this is still a controversial but definitely plausible view (see Childs et al. 2000).

Many management techniques have been proposed to control raccoon rabies. Rabies is a density dependent disease that requires a density threshold necessary for the virus to spread (Carey and McLean 1983). Hanlon et al. (1999) discussed rabies vector population reduction, but because of costs associated with large-scale trapping and shooting campaigns, the possible impacts on non-target species, and lack of public support, this kind of effort is not well accepted. Habitat modification is another proposed technique and includes practices like better refuse management and animal-proofing of

houses, which would reduce exposure rates but would need to be supplemented by a vaccination program. Two such programs are trap-vaccinate-release and oral vaccinations.

The trap-vaccinate-release (TVR) program that involves trapping, vaccinating the animal intramuscularly, and releasing it, seems to be effective on a small scale, but it would prove a cost ineffective strategy for use on a larger scale. TVR control method was used in Ontario in order to prevent an epizootic from crossing over from New York (Broadfoot et al. 2001). Oral rabies vaccination, the distribution of ORV baits that contain an oral rabies vaccine was used to control fox rabies in Europe and seems to be the most promising rabies control technique (Hanlon et al. 1999).

ORAL RABIES VACCINATION

The current ORV baits consist of a fishmeal polymer cube with a plastic sachet that contains the vaccine. A vaccinia-rabies glycoprotein (V-RG) recombinant virus vaccine that has been licensed for oral vaccination of raccoons is also effective in coyotes and foxes (Hanlon et al. 2002). V-RG is the main oral rabies vaccine used in current rabies control programs. The Ohio ORV program was the first large-scale success in the USA. One problem with V-RG is that it is not effective in the oral vaccination of skunks, a major rabies reservoir. Hanlon et al. (2002) tested a new vaccine, SAG-2, on both skunks and raccoons. SAG-2 is a highly attenuated rabies virus vaccine, which, unlike traditional modified live rabies virus vaccines, does not cause rabies when inoculated intramuscularly and intracerebrally in laboratory mice. The SAG-2 vaccine was effective

with both species and was proposed as an alternative to V-RG as safer than the modified-live rabies virus vaccine. Vaccinating both species is important because rabies can continue to spread if one species is vaccinated while the other is not.

To test the attractiveness of ORV baits, Olson and Werner (1999) placed tracking plates next to vaccine-bait units and recorded species-specific visitation and bait uptake rates in Florida. Of the 413 baits, 252 (61%) were contacted by animals and there were no remnants of the baits at 82% of the plates. Raccoons represented 38% of the 252 contacted vaccine-bait units.

Blackwell et al. (2004) evaluated the period of exposure for placebo vaccine baits placed at a density of 75 baits/km^2 relative to raccoon population density. They estimated raccoon density from August to November 2002 and quantified the exposure time of placebo baits from September through October. They found a monthly mean of 24.5 raccoons per km^2, and an average of 84.7% of baits were removed after one week of exposure.

Linhart et al. (2002) tested the attractiveness of flavor-coated sachets between 1996 and 2000. The attractiveness of sachets was tested in part to find a more cost-effective means of delivering the oral vaccine. They compared the flavor coated sachets to the current fish meal polymer baits and found that there was little difference in preference between the flavor coated sachets and the fishmeal polymer baits.

Anderson et al. (1981) discussed the spread of fox rabies across Europe and the population dynamics involved. They present many efforts discussed above and used models to test their effectiveness. According to Dobson (2000) ORV baiting was successful in controlling fox rabies in Europe because fox ecology and movements were

considered in bait development and distribution program design. The baits were focused at the mouths of mountain valleys, a known bottleneck for fox movements. The target application maximized fox exposure to the vaccine and increased the effectiveness of the program.

Hanlon et al. (1998) conducted the first field release of the ORV baits in August of 1990. Their target was the free-ranging raccoon population on Parramore Island, Virginia. Raccoons represented >75% of their bait contacts. They found an overall RVNA sero-prevalence among the raccoons of 52%, but had no estimate of population density.

Beyond the risk to human life, there also is a substantial economic burden of pet vaccination efforts and pre- and post-exposure treatments to people possibly exposed to the virus (Fishbein and Robinson 1993). Estimates for these expenditures in the USA range from $230 million to $1 billion per year. Meltzer (1996) created a general model testing the costs and benefits of the oral vaccination technique for raccoons. Major costs were the purchase and distribution of ORV baits. The benefit of ORV distribution was decreased costs of animal control, laboratory diagnostics, education and administration, and human pre- and post-exposure treatments.

RATIONALE AND SIGNIFICANCE

Although the number of rabies positive raccoons in Ohio decreased after implementation of the ORV program, more information about the program is still needed. Distributing baits at a standard density across large areas of differing land-type use without prior knowledge of the target population could result in unprotected high

density raccoon populations or over-baiting low density populations. Better understanding of raccoon population densities in areas of different land-use types could improve cost efficiency and vaccination efficiency.

Use of pre-bait serological surveys to determine the sero-prevalence and background level titers in a raccoon population to be baited is another important aspect that needs to be incorporated into current ORV programs. This would allow actual estimates of the effect of ORV in a population. Once an effect is known, bait density can be corrected accordingly. The development of an effective and efficient ORV program could result in better use of available funds and help stop the spread of raccoon rabies.

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CHAPTER 2

COMPARISON OF METHODS USED TO ESTIMATE RACCOON POPULATION DENSITY DURING AN ORAL RABIES VACCINE DISTRIBUTION

Abstract: The oral rabies vaccination (ORV) began shortly after the mid-Atlantic raccoon rabies epizootic. The density of the target population is an important consideration in the ORV program, which aims to control the spread of raccoon (*Procyon lotor*) rabies. During April-October (2003 and 2004), I evaluated 3 mark-recapture estimates of raccoon population density, and a line-transect-based method on the 22-km^2 U. S. National Aeronautics and Space Administration Plum Brook Station in Erie County, Ohio (USA; 41° 27' N, 82° 42' W). I calculated annual minimum number known alive (MNKA) density estimates, approximating the protocol used by the U. S. Department of Agriculture Wildlife Services. This method estimated 73 raccoons/km^2 and 68 raccoons/km^2 for 2003 and 2004, respectively. I also calculated adult catch per unit effort (CPUE) estimates, which yielded 50 raccoons/km^2 and 60 raccoons/km^2, respectively for the 2 years. Using program CAPTURE and model M_{bh} (heterogeneity and trap response), I estimated densities of 70 raccoons/km^2 (2003) and 87 raccoons/km^2 (2004). Using program Distance (version 4.1) and my line-transect data, I estimated 9 raccoons/km^2 and 10 raccoons/km^2 for 2003 and 2004, respectively. Line-transect survey

density estimates were less than the number of unique individuals captured during both 2003 and 2004. I note that lack of replication in the MNKA model precludes error estimates. Also, assumptions of equal probability of capture for both the MNKA and CPUE estimates were violated, possibly biasing my estimates low. However, the upper limit of the CPUE estimate in both years was similar to mean estimates from the mark-recapture model. The mark-recapture estimate was most consistent with published density estimates for similar habitats. Although labor intensive, mark-recapture should provide relatively accurate information about population densities in different land-use areas. Further, in situations where trapping would be difficult due to trap exposures (e.g., urban settings), estimates based on line-transect could provide a baseline for estimating the target population density.

Introduction

An oral rabies vaccination (ORV) program designed to control the spread of raccoon (*Procyon lotor*) rabies has been ongoing across the eastern USA since the early 1990s (Rupprecht et al. 1995). The program consists of the aerial and ground distribution of vaccine-laden baits at a density of 75 baits/km^2 (USDA 2003). Between $230 million and $1 billion annually is spent on rabies prevention and post-exposure treatment in the USA alone (Fishbein and Robinson 1993). Approximately $5 million of the annual cost comes from maintaining a 26,268-km^2 ORV barrier spanning from Lake Erie to northeastern Tennessee (Uhaa et al. 1992). However, because differences in patterns of rabies infections can be related to ecology and life history traits of a population (Carey and McLean 1983, Hanlon et al. 1999), the success of similar ORV efforts likely vary among populations (Perry et al. 1989, Johnston and Tinline 2002). Understanding the

relationship between animal population density and the minimum density of ORV baits necessary to confer a sufficient level of herd immunity is a critical component of an effective and cost-efficient immunization program (Rupprecht et al. 1995, Meltzer 1996).

Trapping has traditionally been used to obtain data necessary to estimate raccoon population density (Hoffman and Gottschang 1977, Riley et al. 1998, Rosatte et al. 2001, Prange et al. 2003). Currently, the Wildlife Services (WS) division of the U. S. Department of Agricultures (USDA) uses trapping and a modified removal model, called minimum number known alive (MNKA), to estimate raccoon population density (D. Slate, Wildlife Services, personal communication). Spotlight and road kill surveys also have been used to estimate raccoon population density (Gehrt 2002). Recently, Blackwell et al. (2004) used a forward-looking infrared (FLIR) camera to conduct line-transect surveys and used distance sampling (Buckland et al. 1993) to estimate raccoon population densities. Given the inherent biases with any method of population estimation, as well as the critical aspect of time with regard to response to zoonotic diseases, I examined how methods of density estimation might differ for the same population over the same time period.

My goal was to recommend the most effective method for estimating raccoon population density in the context of an ORV program. My objectives were to 1) estimate the raccoon population density using 3 trapping-based models and a line-transect survey-based method and 2) compare estimates from each method.

Study area

The National Aeronautics and Space Administration (NASA) Plum Brook Station (hereafter referred to as Plum Brook) is located on the Lake Erie coastal plane in northern

Ohio, USA (41°27′N, 82°42′W). The 22-km^2 site, <1 km south of Sandusky in Erie County, consists of 40% herbaceous field, 30% shrubland (*Cornus* spp.) and 30% oak-dominated (~70% *Quercus* spp. and ~5% *Populus* spp.) hardwood forest (Linhart et al. 2002, NASA unpublished data). The site houses active research facilities along with abandoned warehouses, barns, trailers, and sheds. A 2-m high chain link fence topped with barbed wire runs along the 22-km perimeter of Plum Brook. Human access is controlled by a guard station at the entrance to the base that is centrally located on the northern boundary of Plum Brook. Several roads traverse Plum Brook in north-south and east-west directions. Most roads had 20-m mowed grass strips on either side. A patrol road runs alongside the entire perimeter fence. Drainage ditches alongside the roads fill with water when there is abundant rainfall. There also are creeks and ponds throughout Plum Brook that are permanent sources of water. The surrounding areas to the south, east, and west of Plum Brook consist mostly of agricultural land with crops including corn (*Zea mays*), soybean (*Glycine max*), and wheat (*Triticum aestivum*), while the surrounding area to the north is predominantly residential.

Methods

Trapping

I live-trapped and ear-tagged raccoons on Plum Brook during 6 May-16 October 2003 and 30 March-21 October 2004. I trapped within 8 1-km^2 grids that represented the diversity of habitats on Plum Brook (Figure 2.1). One half of each grid (north, south, east, or west) was randomly selected and trapped throughout a season. Ten of 15 possible trap locations, spaced at 250-m intervals throughout the grid, were selected for trapping each week, with a nightly average of 20 traps/km^2 (Figure 2.2). Traps were

rotated in a fixed order between 3 subsets (1 and 2, 1 and 3, 2 and 3) of possible locations, every 4 weeks so that every point was trapped at least twice. The 8 grids were grouped into 4 pairs such that the two areas comprising each pair were separated by ≥1 km at their closest point. I trapped each grid pair for 4 nights and rotated grid pairs each week so that all 8 grids were trapped once every 4 weeks. One rotation through all 8 grids was considered a single trapping period. I completed 6 rotations in 2003 and 7 rotations in 2004.

My trapping effort varied from 10 traps/night during the first week of trapping to 40 traps/night during the last week ($\bar{x}$ = 20 traps/night) in 2003. Trapping effort was constant throughout the season in 2004 (20 traps/night). All traps that captured non-target species or where bait was missing were counted as 0.5 trap-night (Beauvais and Buskirk 1999). All malfunctioning traps were replaced and not counted as a trap-night. All young of the year raccoons captured before September 2003 were too small to tag and were released.

I used single-door, live-catch cage traps (Tomahawk 108.5, 107.0 x 30.0 x 30.0 cm) baited with marshmallows and a 4:3:1 vanilla extract:honey:anise extract mixture. I anesthetized captured raccoons using a 5:1 ketamine:xylazine solution with a dosage of 12 mg/kg following the trapping protocol developed by the Ohio Department of Health (2002). I removed the sedated animal from the trap, checked for presence of ear tags, assessed overall condition, noting any wounds or lesions, and recorded body weight, sex, and age (adult, subadult, juvenile). I estimated age by tooth development and wear, the presence/absence of the penile frenulum for males, and mammary gland development for

females (Lotze and Anderson 1979). I marked any unmarked animals with duplicate tags (Hasco 1005-3), one per ear. I collected blood samples from 139 (35%) and teeth from 85 (21%) captured raccoons during 2003 and blood from 415 (75%) and teeth from 243 (44%) trapped raccoons during 2004. A blood sample and a first premolar were collected for use in a concurrent ORV study (see Chapter 3) while the animal was still under sedation.

I first summarized my data to approximate the MNKA method used by WS to determine raccoon densities in different areas. The WS protocol includes selection of a rectangular or circular 3-km^2 area that is representative of the macrohabitat of the area, with regular boundaries, and preferably with a similar perimeter habitat buffer of 3 km^2. Fifty live-traps, baited with anise/vanilla and marshmallows, are placed opportunistically without clumping throughout the area in order to maximize raccoon captures. Number of animals captured is then equated to either a "low" density estimate (0-2 raccoons/km^2), "standard" density estimate (3-15 raccoons/km^2), or "high" density estimate ($\geq$16 raccoons/km^2) (D. Slate, Wildlife Services, personal communication). For a "standard" density population, trapping is conducted for 10 nights resulting in 500 total trap nights. This technique divides the number of unique individuals captured by the trapping area, which results in a minimum number of unique individuals known alive for a given area. To approximate the WS protocol for a "standard" density population, I averaged number of unique captures per 500 trap-nights across each year. My methods replicated the protocol except that I did not move or remove traps based on numbers of animals captured.

I also analyzed my capture data using a catch per unit effort (CPUE) removal model. The CPUE population and density estimates and their 95% prediction intervals (PI) were calculated by regressing the number of new captures/trap-night during each trapping period on total cumulative captures of novel individuals (White et al. 1982). The CPUE model assumes that every individual in the population would be marked when no new animals are captured. Assuming a closed population, the population estimate is the intercept of the least-squares line with the x-axis ($y = 0$). I used Minitab® 14 for my analysis.

Individual raccoons are known to have unique capture probabilities both before and after the first capture event (Gehrt and Fritzell 1996). Biological information such as this must be considered when selecting an appropriate model (Pollock et al. 1990). Program CAPTURE generates models that estimate population size by relaxing assumptions about time variation, trap response and heterogeneity (Pollock et al. 1990). The most general model M_{tbh}, allows for time variation, heterogeneity and trap response, which makes sense for raccoons when capture events occur over long time spans but this model has no predictor and assumes the relative differences in detection probability among sampling periods are constant (Lee and Chao 1994). Therefore, I used model M_{bh} (trap response and heterogeneity), which includes the jackknife estimator, for my mark-recapture model because the model accounts for both the heterogeneous capture and recapture probabilities and produced a population estimate.

I used only adult captures in my trapping-based population estimates because all 3 methods assume that the population is closed. Adult female raccoons average about 3 kits per year (Ritke 1990). To account for juveniles in the population at the time of

baiting, I added an estimate of 1.5 juveniles/adult to the density estimates. This estimate assumes a 1:1 sex ratio common in raccoon populations (Broadfoot et al. 2001). Departure from 1:1 sex ratio among trapped raccoons was determined with Chi-square tests ($P = >0.05$).

Tracking

A sub-sample of captured adult (>1 year) raccoons were fitted with 130-g radio-collars with mortality switches during a separate concurrent study (Advanced Telemetry Systems, Isanti, MN). Eight males and 9 females were radio-collared during 2003 and 22 males and 22 females were radio-collared during 2004. Raccoons were tracked during 23 September-31 October 2003 and 22 June-23 November 2004. All animals were checked once/week for changes in signal pulse that would indicate mortality (8-hour period of inactivity). Most live radio-collared animals were located 2-4 nights every 2 weeks by triangulation 3-6 times per night between sunset and sunrise (Ellis 1964). A set of 3 or more bearings was obtained on each animal in a 5-min period. Each animal was located at 2 to 3-hr intervals from sunset to sunrise. The Kaplan-Meier method was used to estimate weekly survival rates (Heisey and Fuller 1985).

Triangulation bearings were entered into Locate II, which computed locations of radio-marked raccoons (Nams and Boutin 1991). Universal Transverse Mercator coordinates of raccoon locations were imported into ArcView® 3.2 and the fixed kernel method was used to estimate home-range size using the spatial analysis extension (Worton 1989). A 95% minimum convex polygon that encompassed locations of all radio-collared raccoons was created and used to estimate the area utilized by animals captured on my trapping grids.

Surveys

I conducted line-transect surveys during 6 March-22 October 2003 and 8 March-20 October 2004 to estimate raccoon population density on Plum Brook. In addition to estimates for each 8 month period, I divided observations into 3 time periods: kits in den (March-May), kits foraging with mother (June-July), and kits independently foraging (August-October). I combined the first 2 periods (March-May and June-July) to estimate the population before juveniles were independently foraging and vulnerable to trapping.

I used a vehicle to perform the surveys and used roads as my transect in order to sample the study area in a single night. Surveys were conducted along 5 interior east-west oriented roads, totaling 19.3 km in length (Figure 2.1). The selected roads allowed me to sample the range of habitats present on Plum Brook (Blackwell et al. 2004).

I used a forward-looking infrared camera (FLIR) (Raytheon® Palm IR 250 Digital) connected to a small video screen (Sony® Digital-8 Video Walkman®) to detect raccoons. The camera was mounted on the passenger-side window of a vehicle and its orientation, either north or south, was fixed throughout the survey. I randomly selected the starting point, either north or south, and the direction traveled, either east or west, at the beginning of each survey thus ensuring that I did not sample an area twice. I marked the location where an individual or cluster of raccoons was first detected with a spotlight (Brinkman Q-Beam® Max Million 1,000,000 candle power) then used a laser range finder

(Bushnell® Yardage Pro® 1000) to measure the perpendicular sighting distance from the transect. The geometric center of the cluster was used to measure distance from the transect when a cluster of raccoons was encountered.

I conducted 5 surveys per month, one per week with a second during week three to increase sample size. I started the surveys 30 min after sunset (http://aa.usno.navy.mil). During each survey, I recorded number of raccoons observed, the transect on which they were observed, location on the transect, the perpendicular distance from the transect, and the predominant vegetation where the raccoon was observed (grassland, shrub, or woodland; Belant and Seamans 2000). I did not count any animals disturbed, due to observer presence, before detection or animals that moved onto the transect from the driver's side of the vehicle.

I analyzed the line-transect data using Distance 4.1 (Buckland et al. 1993), which compared the distribution of sighting distances to different models. Akaike's Information Criterion (AIC) was used to select the best fitting model. The estimated area beneath the resulting curve reflected the effective width of the transect, which was used to adjust number of raccoons observed for detectability (Buckland et al. 1993). This function was then used to estimate the raccoon population density with a 95% CI for Plum Brook.

Results

Radio-telemetry

None of the radio-collared raccoons died during 2003 and the Kaplan-Meier survival rate at the end of trapping in 2004 was 75%. Mean home-range size (95% utilization distribution) was 106 ha for females and 157 ha for males. The 95% minimum convex polygon of locations of all radio-marked raccoons captured on my grids during

2003 and 2004 extended beyond most boundaries of Plum Brook (Figure 2.1). Movements outside of the area were primarily due to nocturnal foraging, after which animals returned to Plum Brook. Thus, the area of Plum Brook (22km^2) was the appropriate area for density estimates. The high survival rate and negligible emigration both support the assumption of population closure.

Mark-recapture

Trap success rate during 2003 was 22% and recapture rate was 13%, compared to 31% and 39%, respectively during 2004 (Table 2.1). The male:female sex ratio in my population did not differ from 1:1 in 2003 (M:F ratio = 1.2:1, $\chi^2 = 2.23$, df = 1, P = 0.135) or in 2004 (M:F ratio = 1.2:1, $\chi^2 = 2.09$, df = 1, P = 0.148). The ratio of adults to juveniles for trapped raccoons, after juvenile emergence, was 1.26:1 for both years. Approximately 7% of the animals tagged lost one ear tag during the study. The MNKA adult density estimates were 29 raccoons/km^2 during 2003 and 27 raccoons/km^2 during 2004. Regressing CPUE on cumulative catch produced a fitted regression line with adult population estimates of ($\hat{N}$ ± 95% PI) 438 ± 182 raccoons and 527 ± 208 raccoons (Figure 2.3) for 2003 and 2004 respectively. Estimates of population size from mark-recapture with model M_{bh} were ($\hat{N}$ ± 95% CI) 619 ± 83 during 2003 and 765 ± 92 during 2004. Population estimates were divided by trapping area (22 km^2), then raccoon density at time of baiting was calculated by adding the juvenile adjustment to all trapping based density estimates (Table 2.2).

Line-transect

I observed 273 and 296 raccoons during 2003 and 2004, respectively, over 37 survey nights each year, an average of 5 surveys/month for both years (Figure 2.4).

Juveniles accounted for 7% of the total number of animals detected during 2003 and 6% of the total number of animals detected during 2004. The only surveys with no observations occurred on 6 and 13 March 2003 and only 0.5% of animals observed were not counted due to movement. The group size observed ranged from 1-5 animals, 1 was the most common (mean = 1.3 animals, SD = 0.68). I, therefore, assumed that each raccoon represented an independent data point. Raccoons were sighted 0-196 m (mean = 38-m, SD = 55-m) from the transect during 2003. Sighting distances ranged from 0-145 m (mean = 31-m, SD = 25-m) during 2004. An average of 53% of observations were 0-25 m and 54% were in grass or on paved roads both years. Sighting distances were grouped into 25-m increments and the most distant 5% of the observations were truncated before analysis to remove outliers (Buckland et al. 1993). Grouping of distances has little effect on efficiency and can improve robustness if heaping or movement before detection (i.e. possible response to vehicle) is expected (Buckland et al., 1993).

The model, g(y) = key(y) [1 + series(y)], selected for both years was a uniform key function with a cosine series expansion:

$g(y) = 1/w\left[1 + \sum_{j=1}^{m} a_j \cos\left(\frac{j\pi y}{w}\right)\right]$; cosine adjustments were of orders 1 and 2

(y = detection distance, w = truncation point, a = area of interest) (Table 2.3, Figure 2.5). Density estimates varied among periods and years (Figure 2.6). Density estimates for the pre-juvenile emergence periods (March-July) were 9 ± 3 raccoons/km^2 each year.

Discussion

Using a population of raccoons from northern Ohio as a model, I evaluated 3 trapping-based estimators of population density and a density estimate based on

line-transect methodology. Each method carried different assumptions, which were met to varying degrees. Estimates of the population size based on trapping averaged 3 times higher than those based on the less labor-intensive line-transect surveys.

My decision to use closed population methods to estimate density was supported by the radio-telemetry from trapped raccoons, which showed little movement outside of Plum Brook. There was evidence of some mortality in the population during 2004, which would mean that the population densities were potentially overestimated. However, this does not account for the large difference between mark-recapture and line-transect density estimates.

The 3 basic assumptions of closed population methods are: (1) the population is closed; (2) all individuals in the population have equal catchability; and (3) the probability that an individual is caught is constant throughout all sampling periods (White et al. 1982). In order to achieve a precise estimate using mark-recapture techniques, recapture-rate should be close to 0.5 (White et al. 1982). My average recapture rate (0.26) was relatively low but close to the 0.24 rate reported by Riley et al. (1998).

The low proportion of recaptures indicates heterogeneity and trap response, which violate 2 of the assumptions of closed population estimators. However, because both MNKA and CPUE rely solely on the number of new captures, only the second assumption is violated. The mark-recapture model includes the first assumption but assumes that each animal has its own unique capture probability both before and after the first time it is caught. The MNKA, while logistically practical, provides estimates that do not include any estimate of error around the means. The mark-recapture estimates, as

opposed to those from MNKA and CPUE, were the most reliable for my population because model M_{bh} allows for relaxation of the assumptions of heterogeneity and trap response.

The MKNA method, like CPUE, produced estimates only based on new captures and cannot account for heterogeneity of capture probabilities. The 2003 density estimate from MNKA was close to the mark-recapture estimate but the 2004 estimate was lower. The CPUE density estimates were lower than the mark-recapture estimates in both years.

The MNKA method employed in the WS protocol includes a shortened trapping period taking an average of 10 nights with 50 traps/night. In order to accumulate the same number of trap nights as outlined in the WS protocol I pooled trap nights over 6 weeks. This was the best method available to estimate effort but the longer sampling period could result in larger estimates than would have been obtained in the standard 10-night protocol. The subjective trap placement and movement of "unproductive traps" of the WS protocol will be unrepresentative of a larger area and could produce high density estimates. However, this only occurred in one year of my MNKA estimates compared to mark-recapture. Therefore, MNKA should be used as an index at best. The WS protocol was implemented to estimate raccoon densities across different land use areas. Based on our mark-recapture estimate the MNKA estimate was close but the method does not make use of all of the information that is available from trapping. The CPUE method, while not the best estimate here, could prove to be an improvement over the current use of MNKA in the WS protocol. Catch per unit effort can account for unequal trapping effort and can estimate the population size beyond the actual data, both of which are impossible with MNKA.

The 3 main assumptions of line-transect methodology are: (1) objects directly on the transect line are always detected; (2) objects are detected at their initial location and do not move (or move randomly) before being detected by the observer; and (3) distances and angles are measured accurately (Buckland et al. 1993). A sample size close to 70 is needed to produce precise estimates (Buckland et al. 1993).

While I had some control over the 3 main assumptions during my study, the assumption that lines are randomly located in the study area or that the objects are randomly and independently distributed was not controlled for. Using roads for line-transect surveys is a well known type of convenience sampling (Anderson 2001). Roads and adjacent mowed areas can be unrepresentative of the distinct habitat that often borders them. Alteration of habitat can also affect availability of resources. These two factors can affect raccoon distribution and behavior which in turn will bias detection rates and resulting density estimates (Gehrt 2002). However, use of roads can facilitate sampling across large areas and increase sample sizes.

The change from grass to shrub or forest obstructs visibility just past the mowed strip (>25 m). Visibility in this area of transition might not be as pronounced during surveys conducted earlier in the year, before leaf-out in March-May. Visibility beyond the mowed strips decreases greatly after plants become fully foliated, apparent in the drop in density estimates between May and June (Figure 2.6).

While estimates during the 2 years of my study were consistent, when compared to the preceding study they were low (Blackwell et al. 2004). The only difference in methods between 2002 and 2003-2004 was the observers, otherwise I used the same equipment on the same area over the same transects. When comparing the average

number of observations/survey over the three months Blackwell et al. (2004) found 14, 17 and 16 raccoons/survey for August, September and October respectively and my averages over both years were 9, 8 and 6 raccoons/survey, for the same respective months.

The high percentage of my sightings within 25 m (Figure 2.5) and in grass or on roads has been attributed to these types of surveys before (Blackwell et al. 2004). There also are other factors that vary seasonally and annually such as food availability, water sources, and weather that could affect raccoon distribution along roads within and among years. The differences in visibility along transect widths and violation of the random placement assumption could lead to biased (compared to trapping) population estimates if raccoons move non-randomly with respect to roads. Variation in raccoon movements relative to roads offers a possible explanation for the relatively low density estimates for this method.

Examining my data at 10 m intervals, similar to Blackwell et al. (2004), showed that while most of their observations were in the 0-10 m interval, the largest number of my observations were in the 20-30 m interval(Figure 2.7). This would indicate that there may have been some movement away from the transect. However, the area sampled by the camera was generally in front of the vehicle so animals were more likely detected before they would have reacted to the vehicle. The distance from the vehicle when an animal was first detected, ability to accurately determine the point of initial detection, and the fact that so few animals were removed because of pre-detection movement provides support that there was no direct behavioral response caused by the survey. Further, a drastic change in the size of the raccoon population of Plum Brook between 2002 and

2003 is unlikely but there also should not be such a great difference between methodologically identical studies. Lack of replication in results between studies, and comparison to trapping based density estimates that are similar to published estimates for similar habitats, indicate biases in this modified line-transect method. The inconsistency in results between studies and negative bias in density estimates likely result from some behavioral response of raccoons to the roads used for surveys.

My mark-recapture density estimates of 70 (2003) and 87 (2004) raccoons/km^2 are very similar to density estimates for raccoon populations in suburban environments in the eastern and mid-western United States (Table 2.4). Blackwell et al. (2004) estimated that densities on Plum Brook were 33-14 raccoons/km^2 during summer-autumn 2003. Schinner and Cauley (1974) estimated that their population near Cincinnati, Ohio varied from 11-177 raccoons/km^2 over a year and a half using mark-recapture. Density estimates also ranged from 36-49 raccoons/km^2 in spring (Gehrt 2002) to 58-93 raccoons/km^2 in the fall for a suburban population of raccoons in Illinois (Prange et al. 2003).

Management Implications

From a logistics standpoint, line-transect surveys were less time consuming, averaging about 20 hrs/month compared to 128 hrs/month for trapping. The surveys were also less invasive than trapping. The use of line-transect surveys should, therefore, be considered for estimating raccoons densities in an operational setting. Density estimates from road-based surveys should be equally biased across different undeveloped land-use types which could allow for an estimate of difference in density between

land-use types. Road-based surveys might also be relatively unbiased in urban areas where there is little distinction in habitat between road and adjacent areas. Trade-offs between meeting methodological assumptions and real world applicability should be considered before implementing any method to estimate population densities. Therefore, I assert that long-term trapping data and mark-recapture provided the best density estimate for my raccoon population. But in instances where time and resources are limited CPUE and modified line-transect surveys could provide reasonable estimates of raccoon densities.

The probability of a raccoon rabies outbreak is higher in high density raccoon populations like those found in urban and suburban areas. These are the areas where the efficacy of ORV programs is most important. Distribution of ORV baits at 75 baits/km^2 across different landscapes (urban, suburban and rural) could result in underbaiting high density populations and overbaiting low density populations. Using a density estimate of 125 raccoons/km^2 for an urban area and a density of 6 raccoons/km^2 for a rural area (Table 2.4) the bait densities would range from 625-30 baits/km^2 based on the WS goal of 5 baits/raccoon.

In the case of distribution of ORV as an emergency response to an outbreak, density estimates for similar land-use areas should be used as guidelines for estimating the size of the target population but should not be seen as a replacement for eventual site-specific density estimates. Short-term trapping using CPUE could estimate the target population density and modified line-transect surveys could be used to determine relative

densities across different habitats. With this information effective bait densities could be determined and if there is a limited amount of funding for baits, bait densities for different areas could be redistributed based on resources available.

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Trap Condition	Trap-night assigned	Total trap-nights		Effective trap-nights[a]	
		2003	2004	2003	2004
Capture	1.0	343	390	343.0	390.0
Recapture	0.5	45	154	22.5	77.0
Empty-sprung	0.5	38	84	19.0	42.0
Bait Missing	0.5	47	143	23.5	71.5
Non-target	0.5	24	52	12.0	26.0
Young of the year	0.5	29	45	14.5	22.5
Malfunction	0.0	6	6	0.0	0.0
Empty	1.0	1,350	1,116	1,350.0	1,116.0
Total		1,882	1,990	1784.5	1745.0

[a]Trap-nights adjusted for trap condition.

Table 2.1. Trapping effort and success for raccoons on Plum Brook Station, Ohio during May-October 2003 and March-October 2004.

Method	2003	2004
	$\bar{x}$	$\bar{x}$
MNKA	73	68
Closed mark-recapture	70	87
CPUE	50	60

Table 2.2. Total mean population estimates for minimum number known alive (MNKA), closed mark-recapture, and catch per unit effort (CPUE) for raccoons on Plum Brook Station, Ohio during May-October 2003 and March-October 2004.

Model	Density	95%CI	Ln (likelihood)	K	AIC_c	Δ_i
2003						
Uniform	9	2	-196.93	2	397.93	0.00
Hazard rate	8	2	-196.94	2	397.95	0.02
Half-normal	8	2	-198.95	2	399.93	2.00
Negative exponential	9	2	-196.93	2	399.99	2.06
2004						
Uniform	10	3	-231.65	2	467.36	0.00
Hazard rate	10	3	-231.73	2	467.52	0.16
Half-normal	10	3	-233.34	2	468.70	1.34
Negative exponential	13	4	-232.36	2	468.78	1.42

Table 2.3. Models along with their density estimates, 95% confidence interval (95%CI), log-likelihood (ln [likelihood]), number of estimable parameters (K), Akaike's Information Criterion corrected for small sample bias (AIC_c), and difference (Δ_i) for pooled line-transect surveys conducted on Plum Brook Station, Ohio March-October 2003 and March-October 2004.

Population density (n/km^2)	Landscape type	Source
125	Urban	Riley et al. 1998
94	Urban	Schinner and Cauley 1974
66	Urban	Broadfoot et al. 2001
79	Suburban	This study
73	Suburban	Prange et al. 2003[1]
68	Suburban	Hoffman and Gottschang 1977
42	Suburban	Gehrt 2002[2]
25	Suburban	Blackwell et al. 2004
12	Rural	Gehrt 2002[3]
6	Rural	Prange et al. 2003[4]

[1]Max McGraw Wildlife Foundation, IL, Fall.
[2]Max McGraw Wildlife Foundation, IL, Spring.
[3]Glacial Park, IL, Spring.
[4]Glacial Park, IL, Fall.

Table 2.4. Published estimates of raccoon population densities by landscape type in eastern North America.

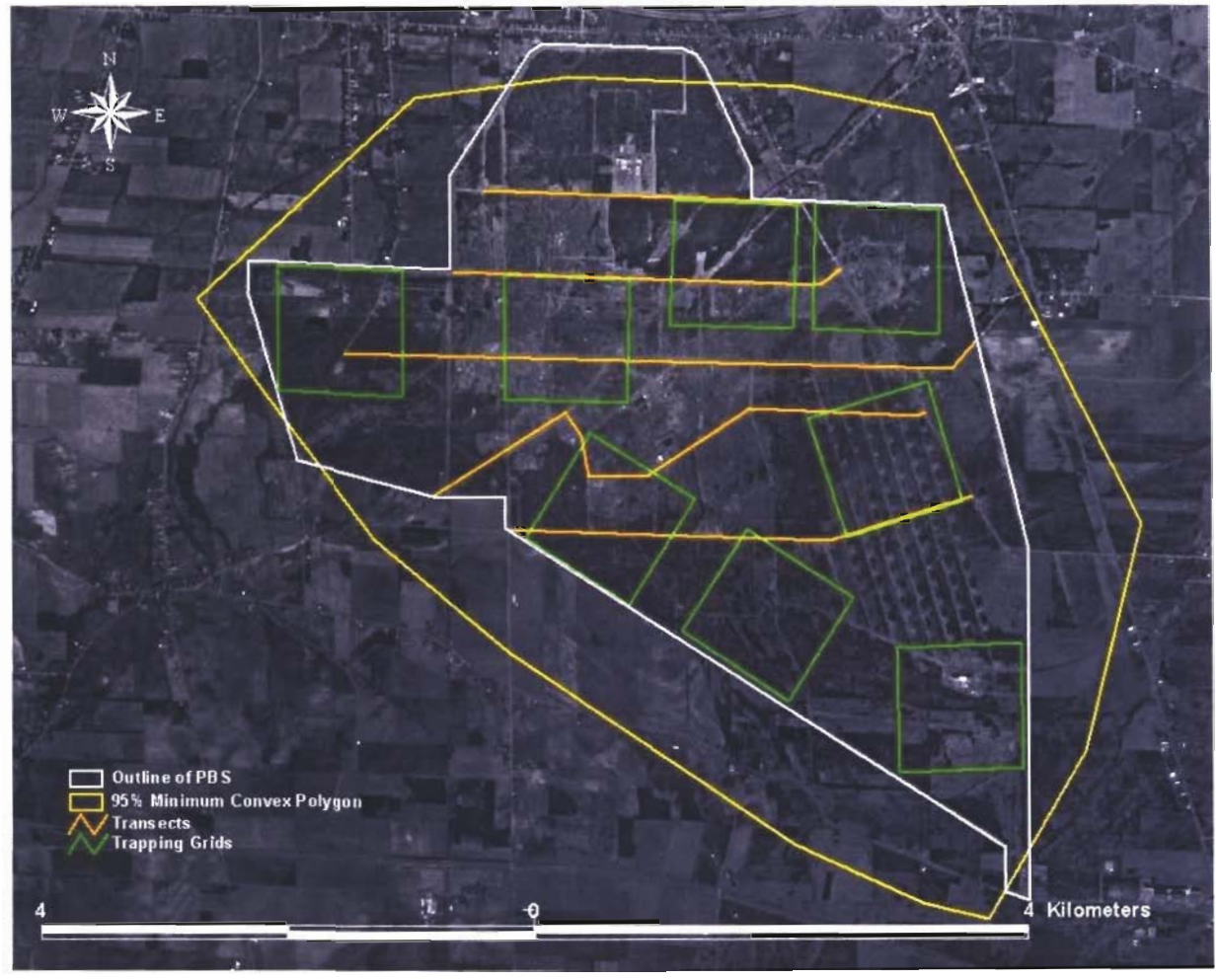

Figure 2.1. Map of 22-km^2 Plum Brook Station (PBS), Ohio with area outline, 1-km^2 trapping grids, transects and minimum convex polygon for raccoons tracked during 2003 and 2004.

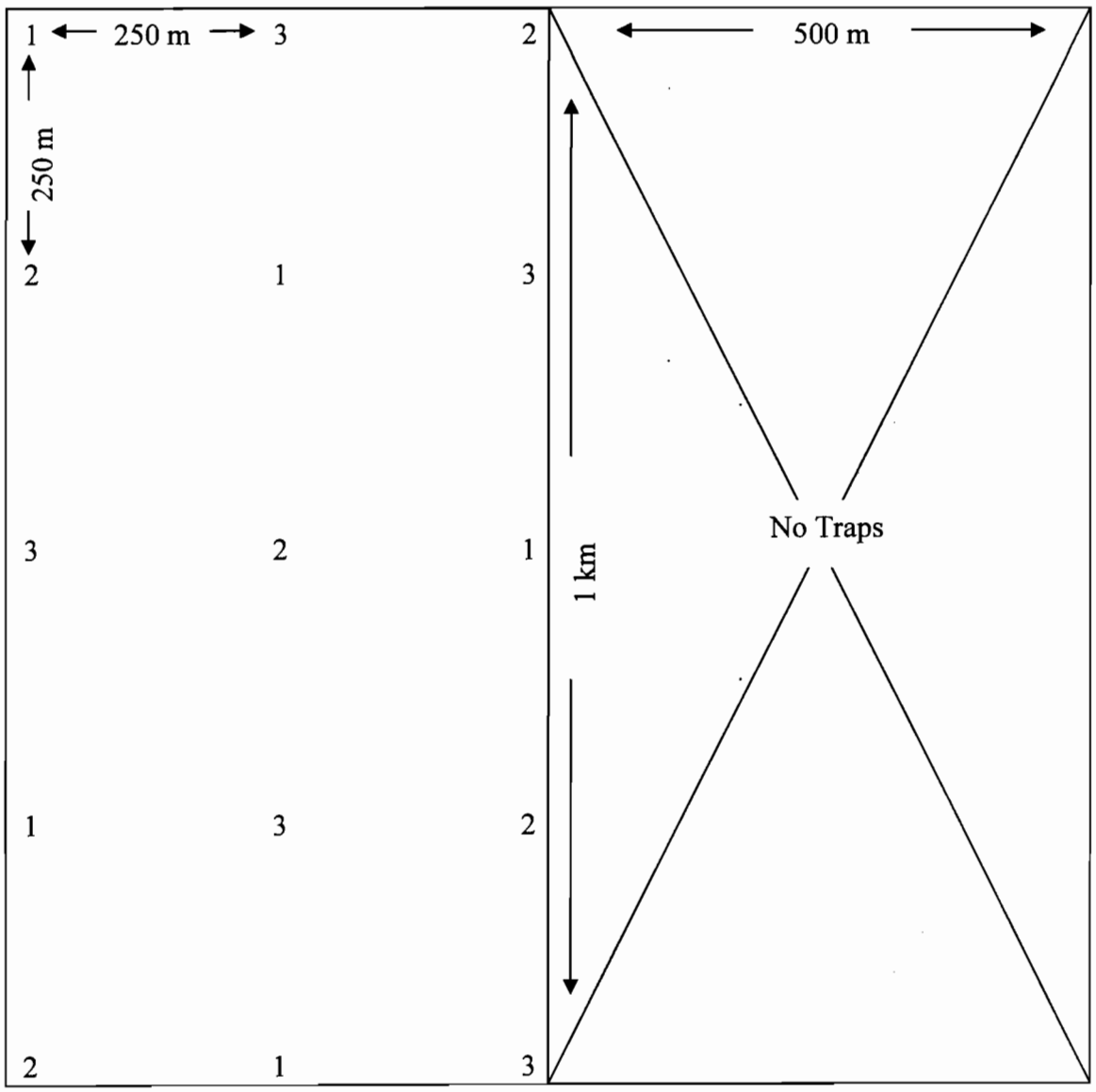

Figure 2.2. Possible trap locations and spacing within the 1-km^2 trapping grid used for trapping raccoons on Plum Brook Station, Ohio during 2003 and 2004.

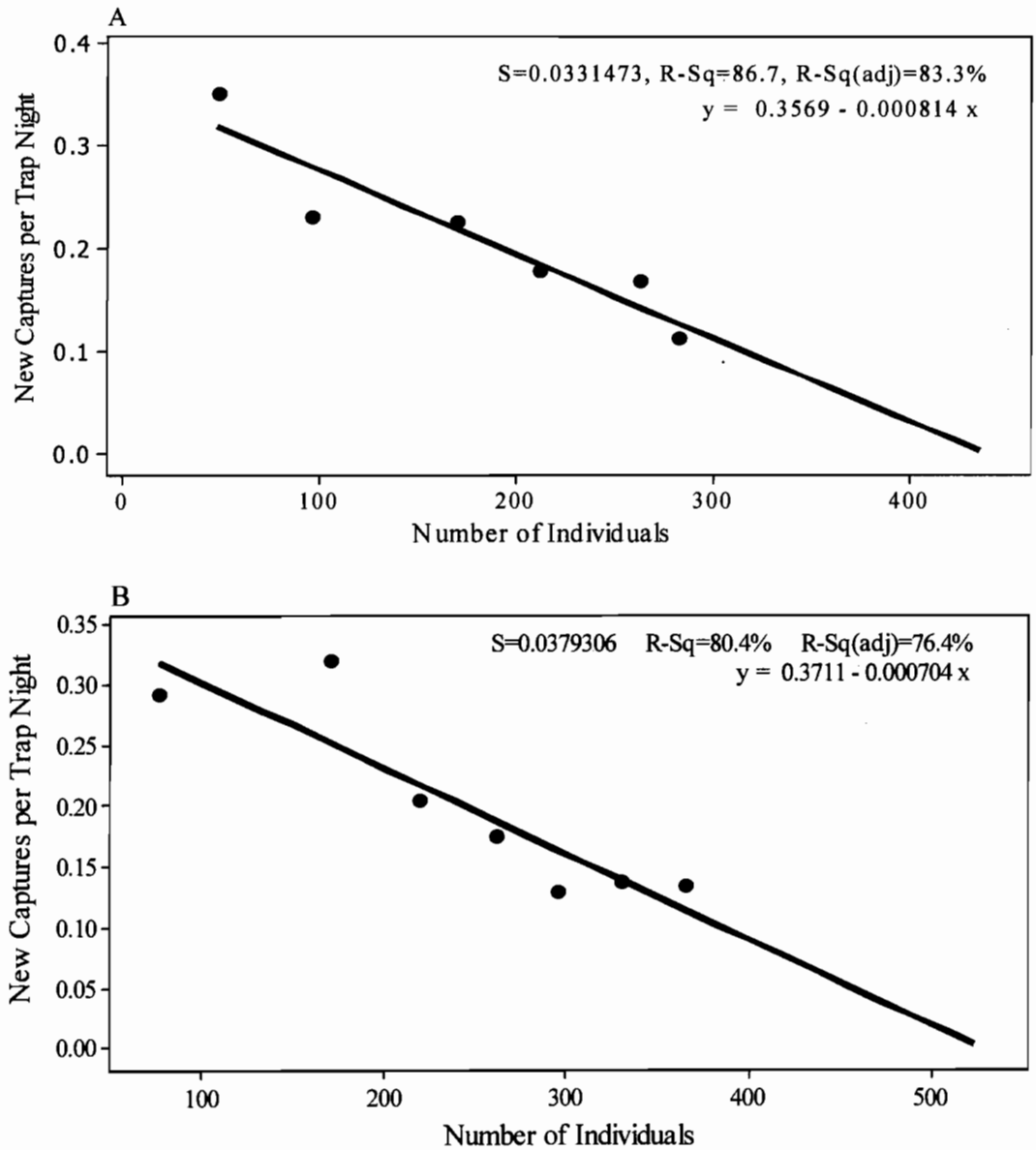

Figure 2.3. Catch per unit effort regression for live-trapped adult raccoons on Plum Brook Station, Ohio May-October (A) 2003 and (B) 2004.

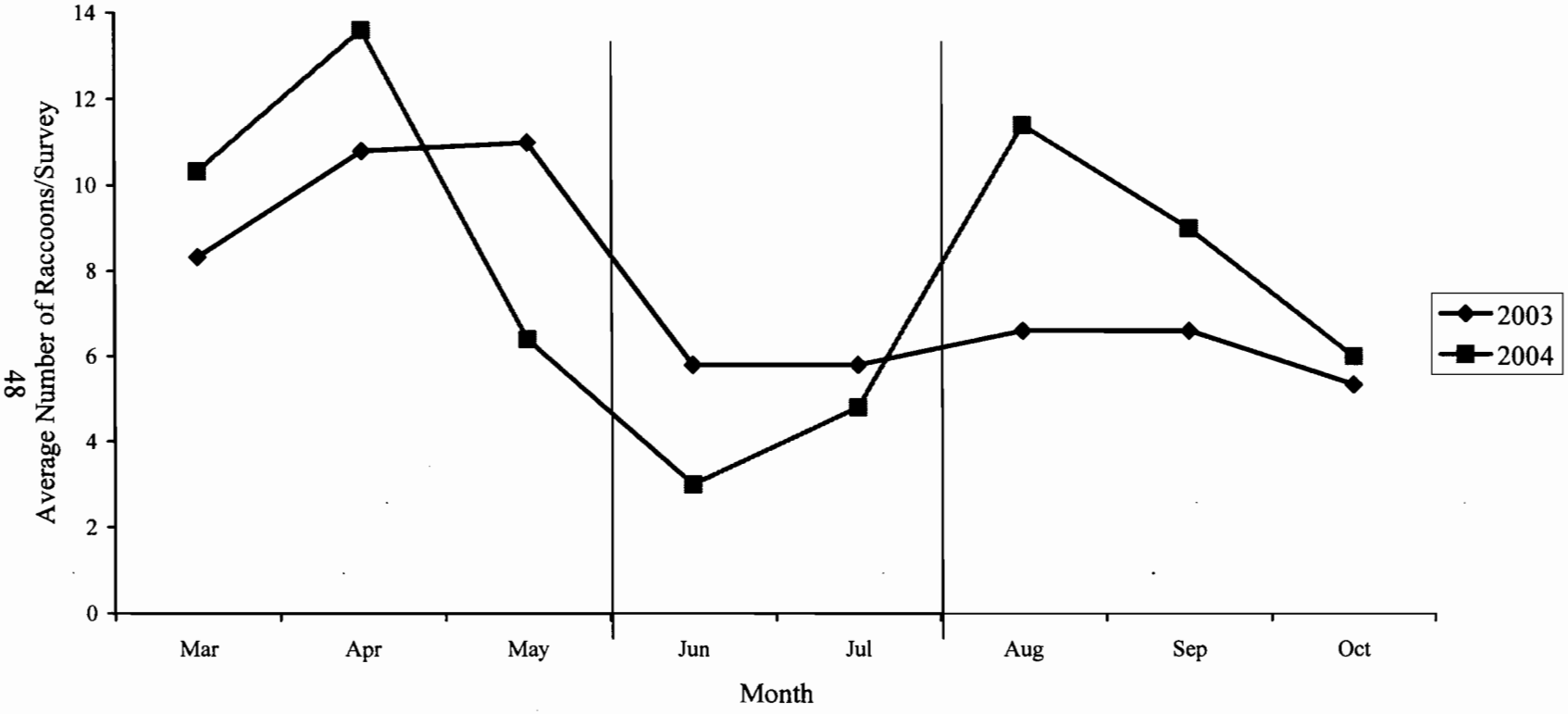

Figure 2.4. Average number of raccoons detected per survey by month for line-transect surveys using a forward looking infrared camera on Plum Brook Station, Ohio March-October 2003 & 2004.

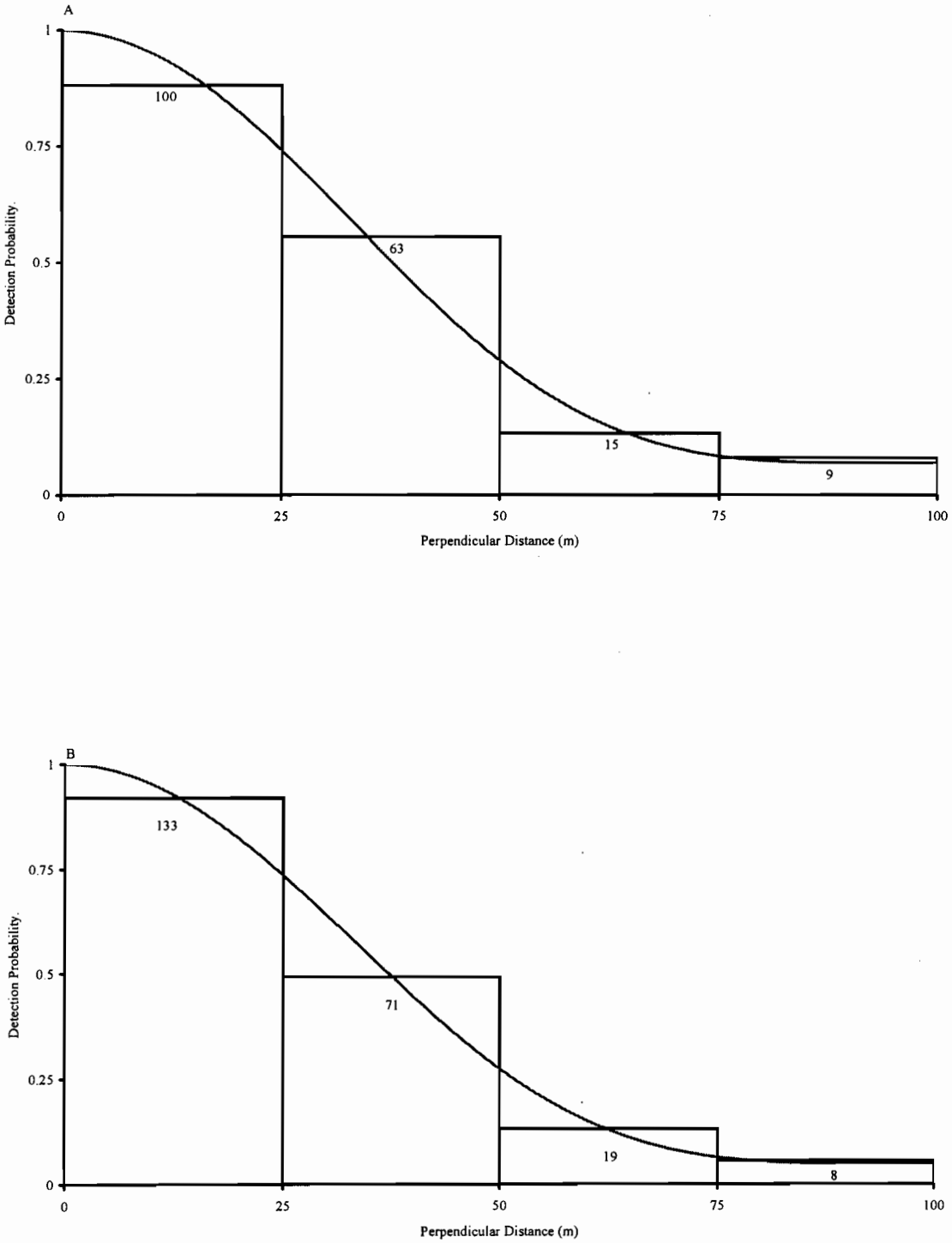

Figure 2.5. Detection function and probability by perpendicular distance (m) off transect for raccoons during entire season of line-transect surveys on Plum Brook Station, Ohio March-October (A) 2003 and (B) 2004.

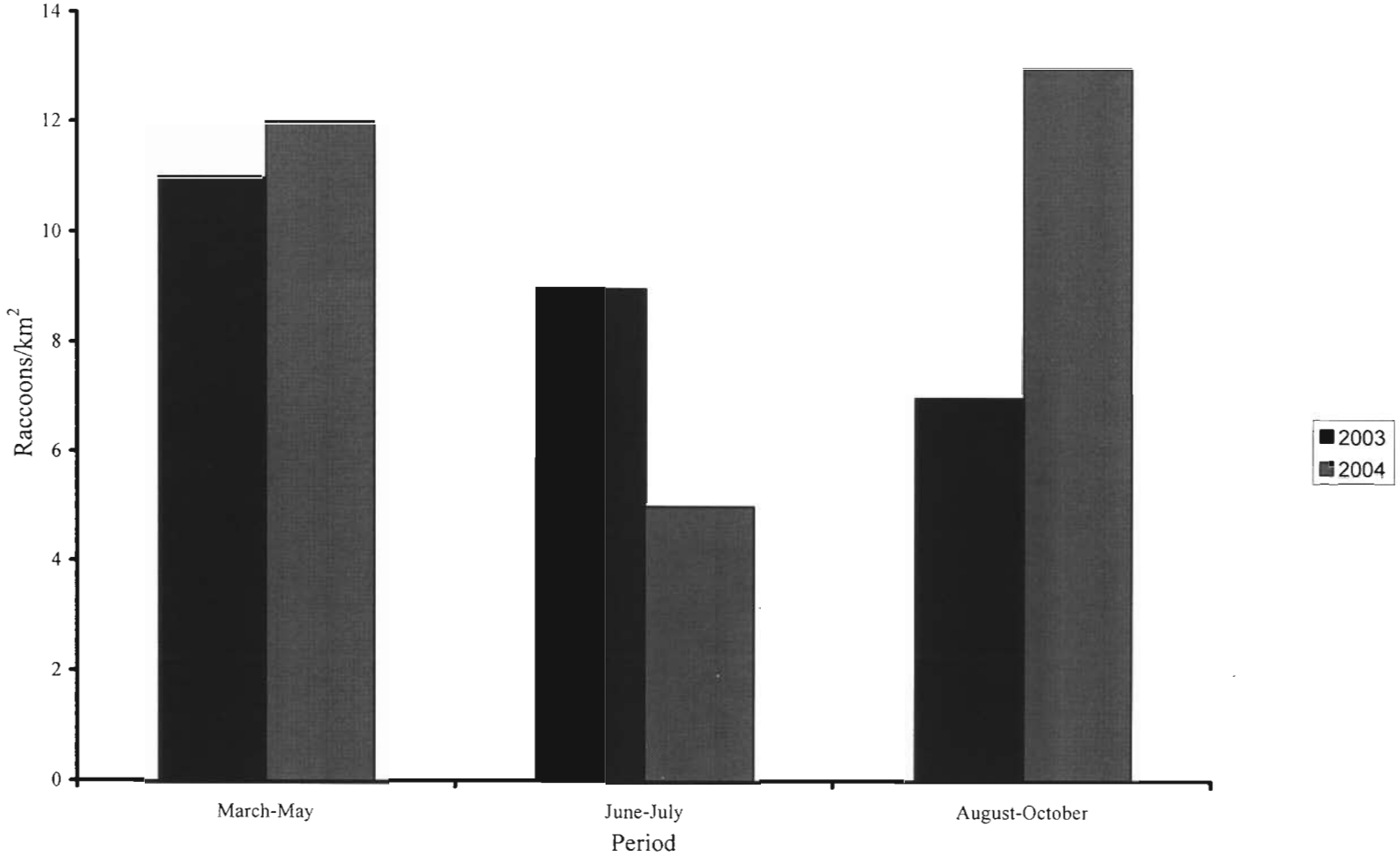

Figure 2.6. Density of raccoons by period for line-transect surveys conducted on Plum Brook Station, Ohio March-October 2003 and 2004.

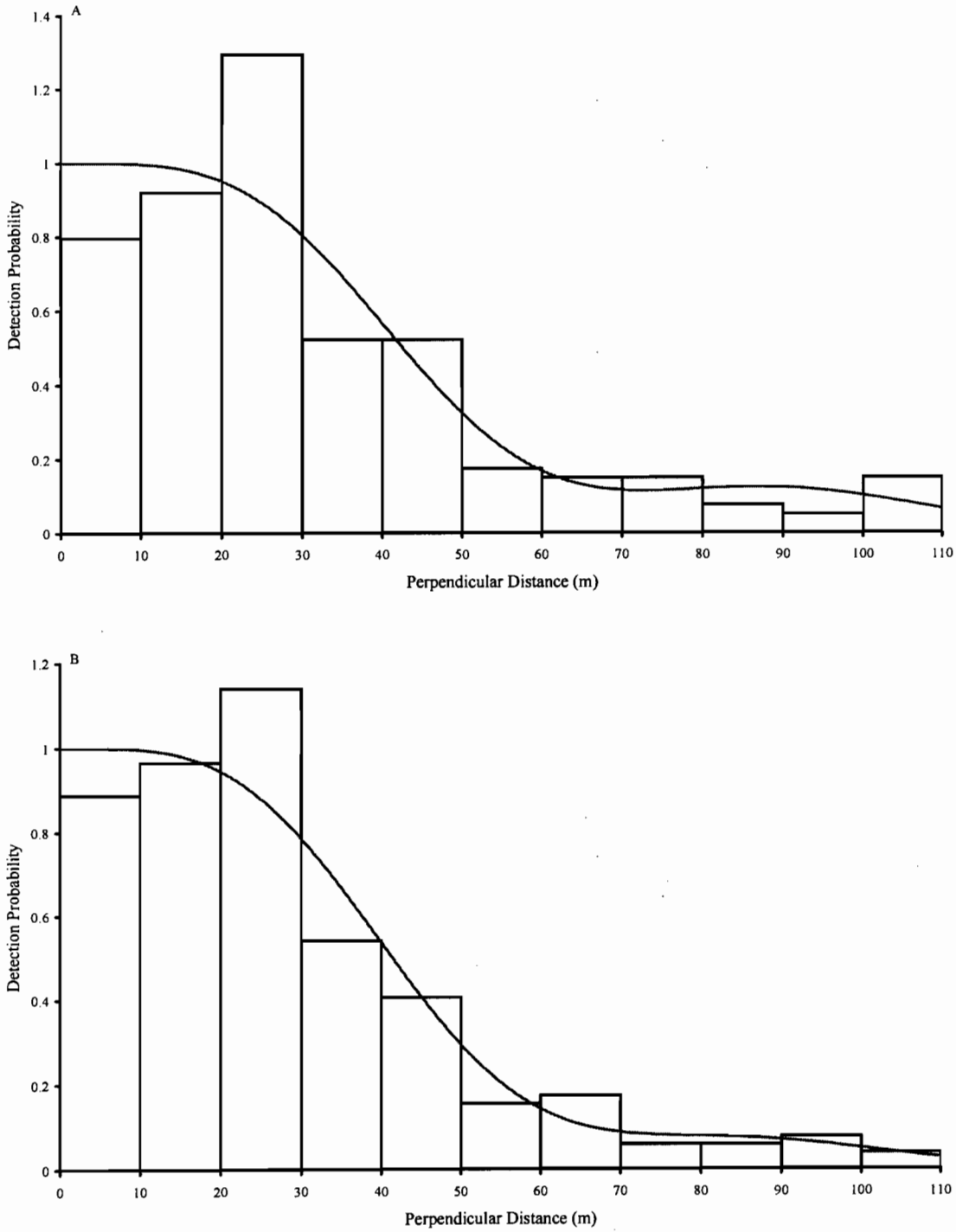

Figure 2.7. Detection function and probability by perpendicular distance (m) off transect (as per Blackwell et al. 2004) for raccoons during entire season of line-transect surveys on Plum Brook Station, Ohio March-October (A) 2003 and (B) 2004.

CHAPTER 3

IMPORTANCE OF SEROLOGY AND POPULATION DENSITY ESTIMATION IN A NORTHERN OHIO RACCOON POPULATION BEFORE THE DISTRIBUTION OF ORAL RABIES VACCINES

Abstract: Current oral rabies vaccination (ORV) programs were established to prevent the westward spread of the raccoon (*Procyon lotor*) rabies virus. The program distributes vaccine-baits at a density of 75 baits/km^2. However, few studies have examined the relationship of bait density and population density to sero-prevalence of rabies virus-neutralizing antibodies (RVNA). I conducted experimental baitings in August 2003 and 2004, 150 km west of the ORV zone (Sandusky, Ohio) where there was no history of raccoon rabies. I collected blood samples from live-trapped raccoons to determine sero-prevalence of RVNA, and teeth to determine prevalence of tetracycline (biomarker in bait). A closed population mark-recapture model was used to estimate the size of the target population. During 2003, 41% of 37 pre-bait serum samples were RVNA positive (≥0.05 IU/ml), but none had titers ≥0.25 IU/ml. During the pre-2004 bait drop period (March-August), 21% of 315 samples collected were RVNA positive and 9% had titers ≥0.25 IU/ml. Although tetracycline prevalence in teeth indicated that 17-27% of raccoons ingested baits, only 4% of serum samples collected after bait distribution

(September-October) had titers ≥0.25 IU/ml in 2003 and 2004. The closed population mark-recapture estimate of adult population size was 619 ± 83 (95% CI) raccoons in 2003. Assuming an annual birthrate of 1.5 juveniles per adult, 1,548 raccoons were present at the time of the 2003 baiting. Compared to the 1,544 baits distributed, just under 1 bait was distributed per raccoon, well below the ORV program target of 5 baits per raccoon. The presence of RVNA before baiting and the decline in sero-prevalence could be attributed to a seasonal exposure to an enzootic non-raccoon strain rabies virus or to the raccoon strain rabies virus that is not known to be enzootic in this area. The high proportion of RVNA positive raccoons in an area with no history of raccoon rabies or vaccination efforts establishes the need for pre-bait serology in order to accurately measure change in sero-prevalence of RVNA after ORV distribution. Without incorporating pre-bait serology and population density estimates, an ORV program could overestimate the number of vaccinated animals and result in under-baiting of high density populations.

INTRODUCTION

Rabies is a zoonotic virus that, if contracted, causes encephalitis and eventual death (Rupprecht et al. 1995). The virus is spread primarily via contact with an infected animal's saliva, through a bite or contact with a mucous membrane or open wound. Rabies is a disease of great concern because it can infect many host species, including humans. There were about 50 human rabies cases per year before canine rabies was controlled in the USA during the 1950s (Fishbein and Robinson 1993). Since then, reported human rabies cases contracted in the USA dropped to only 8 during 1980-1993, with most of these attributed to bat rabies. An outbreak of the raccoon strain rabies virus

in the eastern USA in the 1980s increased concerns about human health (Dobson 2000). Costs associated with rabies control and prevention were estimated to increase by more than 2.5 times after a raccoon rabies epizootic (Uhaa et al. 1992). Since the outbreak, between $230 million and $1 billion per year is spent on rabies prevention and post-exposure treatment in the USA alone (Fishbein and Robinson 1993).

Development of an ORV to combat the raccoon strain rabies virus began shortly after the mid-Atlantic outbreak was detected. A vaccinia-rabies glycoprotein (V-RG) recombinant virus vaccine was found to be efficacious in producing a detectable positive RVNA titer (≥0.05 IU/ml) in a laboratory setting (Rupprecht et al.1988). The V-RG ORV is made by inserting the gene coding for the rabies virus glycoprotein into vaccinia, a living poxvirus vector. Replication of the V-RG recombinant virus in the raccoon, results in rabies virus glycoprotein production that the raccoon immune system recognizes as foreign. This then stimulates the hosts antibody mediated immune response to the rabies virus glycoprotein, producing RVNA.

Large-scale rabies control efforts were stimulated by the ongoing mid-Atlantic epizootic of raccoon rabies detected in the 1980s (Rupprecht et al. 1995). One approach, aerial distribution of ORV, has been ongoing across the eastern USA since the early 1990s, and in Ohio since 1997. The current Appalachian ridge ORV barrier extends from Lake Erie south through northern Tennessee (Figure 3.2). Combining knowledge of bait density and distribution with the knowledge of seasonal raccoon population densities and movements could improve the effectiveness of the current barrier.

The current Ohio ORV program includes cooperation and funding from state (Ohio Departments of Health [ODH] and Natural Resources [ODNR]) and federal

agencies (US Department of Agriculture [USDA] and Centers for Disease Control and Prevention [CDC]). The ORV program began in Ohio as a response to a raccoon rabies epizootic that resulted in 59 rabies-positive raccoons during 1997 (ODH 2002). There were no reported cases of rabies-positive raccoons in Ohio 3 years after establishing the ORV barrier on the eastern edge of the state (ODH 2005). However, another outbreak occurred during 2004 in northeastern Ohio, resulting in 45 known cases of rabies-positive raccoons (Figure 3.1).

The Ohio Department of Health measured prevalence of RVNA in raccoon sera after ORV baits were distributed at 3 densities (ODH 2002). However, background levels of RVNA were not measured and the size of the target population was not estimated. Sero-prevalence of RVNA due to ORV distribution could have been overestimated without a pre-bait estimate. Lack of a density estimate for this area prevents any indication of number of baits distributed per raccoon.

The goal of this study was to measure the change in sero-prevalence of RVNA in a raccoon population of a known density, after distribution of ORV baits at a density of 75 baits/km^2. My objectives were to 1) determine sero-prevalence of RVNA in the population before distribution of ORV, 2) estimate raccoon population density within the area, 3) distribute ORV baits following standard operational protocol, and 4) determine RVNA sero-prevalence after ORV distribution. I expected that RVNA would not be present in the population before I distributed baits and that there would be an increase in sero-prevalence of RVNA in the population after the distribution of ORV baits at an

appropriate density for this population. I also predicted titer levels to increase from the 2003 post-bait to the 2004 post-bait, with the chance for a second exposure to the vaccine.

STUDY AREA

I conducted my study on the National Aeronautics and Space Administration (NASA) Plum Brook Station (hereafter referred to as Plum Brook). The 22-km^2 Plum Brook Station is located within the Lake Erie coastal plane in northern Ohio, USA, <1 km south of Sandusky in Erie County (41°27′N, 82°42′W). Habitat on Plum Brook consists of 40% herbaceous field, 30% shrubland (*Cornus* spp.) and 30% oak-dominated (*Quercus* spp. and *Populus* spp.) hardwood forest (Linhart et al. 2002, NASA unpublished data). The site houses active research facilities along with abandoned warehouses, barns, trailers, and sheds. A 2-m high chain link fence topped with barbed wire runs along the 22-km perimeter of Plum Brook. Human access is controlled by a guard house that is centrally located on the northern boundary of Plum Brook. Several roads traverse Plum Brook in north-south and east-west directions, most with 20-m mowed grass strips on either side. A patrol road runs alongside the entire perimeter fence. Drainage ditches alongside the roads fill with water when there is abundant rainfall. There also are creeks and ponds throughout Plum Brook that provide permanent sources of water. The surrounding area to the north is predominantly residential, while the surrounding areas to the south, east, and west of Plum Brook consist mostly of agricultural land. The crops grown include corn (*Zea mays*), soybean (*Glycine max*), and wheat (*Triticum aestivum*).

METHODS

Oral Rabies Vaccine

The Raboral V-RG® (Merial) ORV baits used in this study are also currently used by the state and federal rabies control programs and are licensed for oral vaccination of raccoons (Hanlon et al. 2002). The bait is a hollow cube of fishmeal polymer that is used to attract raccoons to the ORV. The plastic sachet that contains the vaccinia-vectored rabies vaccine is sealed in the bait with wax. The sachet must be punctured to release the vaccine.

Tetracycline, a biomarker mixed with the fishmeal polymer during manufacturing, is used to detect bait ingestion (Nunan et al. 1994). Tetracycline in the bait chelates with calcium ions in bone and teeth and fluoresces under ultraviolet light. Year of tetracycline deposition was determined by its relative position to annular rings in the tooth. The compound is uncommon in nature and, thus, serves as an indicator of bait ingestion (Linhart and Kennelly 1967). However, presence of tetracycline does not indicate exposure to the vaccine or development of RVNA. The poxvirus, on the other hand, can be used as an indicator of serological response to the vaccine because animals exposed to the vaccine also develop poxvirus-neutralizing antibodies (PNA). Sero-conversion of RVNA must still be measured directly from blood samples collected from animals.

Trapping

I live-trapped and ear-tagged raccoons on Plum Brook during 6 May-16 October 2003 and 30 March-21 October 2004. I trapped within 8 1-km^2 grids that represented the diversity of habitats on Plum Brook (Figure 3.3). One half of each grid (north, south,

east, or west) was randomly selected and trapped throughout a season. Ten of 15 possible trap locations, spaced at 250-m intervals throughout the grid, were selected for trapping each week, with a nightly average of 20 traps/km^2. Traps were rotated in a fixed order between 3 subsets of possible locations every 4 weeks so that every point was trapped at least twice. The 8 grids were grouped into 4 pairs such that the two areas comprising each pair were separated by ≥1 km at their closest point. I trapped each grid pair for 4 nights and rotated grid pairs each week so that all 8 grids were trapped once every 4 weeks. One rotation through all 8 grids was considered a single trapping period. I completed 6 rotations (4 pre-bait and 2 post-bait) during the 2003 field season and 7 (5 pre-bait and 2 post-bait) during 2004.

My trapping effort varied from 10 traps/night during the first week of trapping to 40 traps/night during the last week ($\bar{x}$ = 20 traps/night) in 2003. Trapping effort in 2004 was constant throughout the season (20 traps/night). All traps that captured non-target species or where bait was missing were counted as 0.5 trap-night (Beauvais and Buskirk 1999). All malfunctioning traps were replaced and not counted as a trap-night. All young of the year raccoons captured before September 2003 were too small to tag and were released.

I collected blood samples from an area outside of Plum Brook because of previous ORV studies conducted on the area (Linhart et al. 2002, Blackwell et al. 2004), and presence of pre-bait RVNA in 2003. Therefore, I trapped raccoons at Old Woman Creek National Estuarine Research Reserve, Huron, OH, 15 km east of Plum Brook (41°22′N, 82°31′W) during 2004. Five traps were placed opportunistically on the area each night, once per week throughout the season.

I used single-door, live-catch cage traps (Tomahawk 108.5, 107.0 x 30.0 x 30.0 cm) baited with marshmallows and a 4:3:1 vanilla extract:honey:anise extract mixture. I anesthetized each captured raccoon using a 5:1 ketamine:xylazine solution with a dosage of 12 mg/kg following the trapping protocol developed by the Ohio Department of Health (2002). I removed the sedated animal from the trap, checked for presence of ear tags, assessed overall condition, noted any wounds or lesions, and recorded body weight, sex, and age (adult, subadult, juvenile). I estimated age by tooth development and wear, the presence/absence of the penile frenulum for males, and mammary gland development for females (Lotze and Anderson 1979). I marked any unmarked animals with duplicate tags (Hasco 1005-3), one per ear.

Blood Collection and Analysis

I collected a blood sample from the jugular vein of trapped raccoons after assessing the animal's general condition. The ventral portion of the neck was shaved and cleansed with alcohol. Blood samples (approximately 10 ml) were extracted with a 21 gauge 4-cm needle attached to a vacuum tube. I then recorded the identification number and collection date on the tube and stored it in a cooler until it was centrifuged. I centrifuged (Clay Adams Dynac Centrifuge 420101) the blood for 20 min at 800xg then removed the serum and divided it among three cryovials, each containing $\geq$0.5 ml. All samples were stored at –20 C. Two samples were kept in reserve and one was shipped to the CDC to be analyzed for RVNA titer, via the rapid fluorescent focus inhibition test (RFFIT) (Reagan et al. 1983). Serum samples collected in 2003 were also analyzed by the CDC for PNA titer, via the enzyme-linked immunosorbent assay (ELISA) (Marennikova et al. 1981).

Tooth Extraction and Tetracycline Analysis

I extracted a first premolar from captured animals. The first premolar is a single-rooted tooth that is absent in some individuals. No tooth sample was taken from individuals lacking first premolars. Teeth were collected before and after ORV distribution to establish a baseline for distinguishing between tetracycline deposited during my study and past ORV experiments on Plum Brook (Linhart et al. 2002, Blackwell et al. 2004). Tooth samples were also used to determine the age of the animal. I extracted the first premolar while the animal was still sedated using a jaw-spreader that fit between the upper and lower canines on one side of the mouth and a dental elevator and extraction forceps to remove the tooth. I flushed the site with antiseptic after the tooth was extracted and covered the wound with gauze. I placed the tooth inside an envelope labeled with the animal's identification number and date of collection. Teeth were sent to Matson's Laboratory, LLC where they were cross-sectioned and viewed under magnification to detect tetracycline using an ultraviolet illumination microscope. Age was determined by counting cementum annuli (Nunan et al. 1994).

Density Estimation

Mark-recapture was used to estimate the density of the adult raccoon population present on Plum Brook during my study. Using program CAPTURE, Model M_{bh} (heterogeneity and trap response) (Pollock et al. 1990), a closed population estimate based on trapping data was found to be the most reliable estimate after comparing

methodologies and the preservation or violation of assumptions for each (Chapter 2). To account for juvenile presence in the population at the time of baiting, I added an estimate of 1.5 juveniles/adult to the density estimate (Ritke 1990).

Experimental Baiting

I conducted an experimental baiting designed to replicate the operational baiting conducted by Wildlife Services from fixed wing aircraft on 26 August 2003 and 19 August 2004. I followed the Wildlife Services protocol except that ORV baits were distributed via Bell Jet Ranger® helicopter in place of fixed-wing aircraft. I used a helicopter because of the relatively small size of my study area ($22km^2$). The target bait density for operational baitings is 75 baits/km^2 (ODH 2002). The operational baiting protocol calls for no baits to be distributed over water or buildings and additional baits are usually distributed by hand around such areas. No additional hand-baiting was conducted during this study.

I distributed baits over 14 southeast-northwest oriented flight lines (30-227 baits/line), with an estimated 27-m spacing of baits along the line. Flight lines were spaced 0.5 km apart (Figure 3.3) and the helicopter traveled at a speed of 80 km/hr, at an altitude of 150 m above ground. Trapping was suspended for 1 week after bait distribution to allow time for animals to contact the ORV baits.

Bait Viability Test

I tested the viability of the vaccine with a sample of ORV baits from the baits available for distribution in 2003. I placed 10 baits each into 2 wire traps (to prevent consumption) immediately following the 2003 bait drop. Both traps were exposed to ambient conditions but one was exposed to direct sunlight, while the other was shaded.

A third group of 10 baits was refrigerated at 3 C as a control. All baits were collected at the end of trapping (7 weeks) and sent to the CDC for viral vaccine titer measurement using cell culture (Rupprecht et al. 1988).

Statistical Tests

I used Chi-square to test independence of RVNA and PNA prevalence in individual raccoons ($P > 0.05$). Chi-square was also used to test independence of RVNA and tetracycline. I also calculated odds ratios which express the likelihood of a raccoon being RVNA positive when it is PNA positive. Odds ratios were also used to express likelihood of being RVNA positive when tetracycline positive.

All animal handling procedures followed protocol 2003A0119, approved by the Ohio State University (OSU) Institutional Laboratory Animal Care and Use Committee (ILACUC) and reviewed by the Institutional Animal Care and Use Committee convened by the U. S. Department of Agriculture, Animal Plant and Health Inspection Service, Wildlife Services, National Wildlife Research Center.

RESULTS

Population Density and Bait Density

The total population estimate of raccoons on Plum Brook was 1,548 raccoons during 2003 and 1,913 raccoons during 2004. The total off-time during the bait drops for Plum Brook amounted to about 6% of the total area. The realized bait density was 72 baits/km^2 (1,544 baits) after accounting for the off-time over buildings and water.

Tetracycline, PNA and RVNA

Although no teeth in the 2003 pre-bait (May-August) sample were tetracycline positive, I detected a high proportion of low positive titers ($0.05 \leq$ -<0.25 IU/ml) of

RVNA and PNA before distribution of ORV baits (Table 3.1). Only 8% of serum samples were RVNA positive after the bait drop (September-October), but half of these samples exhibited titers ≥0.25 IU/ml. Twenty percent of the post-bait serum samples were positive for PNA, but 75% of these were <0.25 IU/ml. Prevalence of tetracycline (Table 3.3) in the 2003 post-bait sample indicated that 17% of the population ingested baits. Prevalence of RVNA differed between PNA positive and PNA negative serum samples both before ($\chi^2 = 0.08$, df = 1, P = 0.784) and after baiting ($\chi^2 = 1.99$, df = 1, P = 0.159) in 2003. Prevalence of RVNA differed between tetracycline positive and tetracycline negative serum samples during the 2003 post-bait ($\chi^2 = 1.58$, df = 1, P = 0.791). Poxvirus-neutralizing antibody positive raccoons were only 1.24 times more likely to be RVNA positive before the bait drop in 2003, but they were 2.89 times more likely to be RVNA positive after the 2003 bait drop (Table 3.2). A tetracycline positive individual was 2.93 times more likely to be RVNA positive after the 2003 bait drop (Table 3.4).

Prevalence of RVNA was 21% in the 2004 pre-bait period (April-August), with less than half of the samples exhibiting titers ≥0.25 IU/ml (Table 3.1). The proportion of RVNA positive animals again declined after the bait drop in 2004 (September-October) with only 9% of samples RVNA positive and less than half of those samples exhibiting titers ≥0.25 IU/ml (Figure 3.4). Tetracycline results indicated that 27% of the population ingested baits after the 2004 bait drop. Prevalence of RVNA differed between tetracycline positive and tetracycline negative serum samples during the 2004 post-bait ($\chi^2 = 0.61$, df = 1, P = 0.436). A tetracycline positive individual was 1.79 times more likely to be RVNA positive after the 2004 bait drop (Table 3.4).

Five of 36 (14%) serum samples collected at Old Woman Creek during 2004 were positive for RVNA, but all titers were <0.12 IU/ml. The highest percentage of positive samples were observed in May (38%, $n = 8$), followed by June (17%, $n = 6$), and July (13%, $n = 8$). No positive samples were collected from Old Woman Creek during April, August or September.

I collected 2 or more serum samples from 2 RVNA positive individuals during 2003 and from 12 RVNA positive individuals during 2004 (Table 3.5). Three individuals had one serum sample collected during 2003 and one during 2004. Of the RVNA positive individuals with 2 or more serum samples collected, 59% of titers declined over time, but only 18% increased after distribution of ORV. Also, 18% of titers increased either between the 2003 post-bait and the 2004 pre-bait or within the 2004 pre-bait period.

Bait Titers

The geometric mean titer (GMT) of the V-RG virus in the refrigerated baits was 9.0 $\log_{10}$ Tissue Culture Infectious Dose ($TCID_{50}$)/ml (8.2-9.2 $\log_{10}$ $TCID_{50}$/ml), and the GMT for the shaded baits was 7.2 $\log_{10}$ $TCID_{50}$/ml (<5.2-9.2 $\log_{10}$ $TCID_{50}$/ml). No virus was detected in any of the exposed baits. The mean daily temperature during the bait exposure experiment was 16 C with a mean daily maximum of 22 C and mean daily minimum of 11 C. Temperatures ranged from 33 C on 25 August to 0 C on 6 October.

DISCUSSION

Serum samples collected from raccoons before and after distribution of ORV baits showed that there was some effect of the simulated operational ORV baiting. However, low contact rates and a relatively high raccoon density indicated that the bait density was well below the operational goal of 5 baits per raccoon for this population.

Pre-bait Titers

My results showed that a high proportion of the population had positive RVNA titers before distribution of ORV baits. I also found that sero-prevalence of RVNA actually declined after the bait distribution. The notable aspect of my results is that this occurred 150-km outside of the known raccoon rabies enzootic area. The multiple serum samples collected from positive individuals also suggested exposure to a rabies virus antigen from a source other than our distributed ORV which caused development of RVNA in raccoons. Some possible explanations include previous vaccination, translocation of infected animals or possibly a non-fatal infection from exposure to a strain of non-raccoon rabies virus.

The high pre-bait sero-prevalence of RVNA might be expected from a large scale trap-vaccinate-release program. However, no local veterinarians or rehabilitators contacted had any knowledge of anyone in the area vaccinating and releasing raccoons. The uniform distribution of RVNA positive animals across the 22-km^2 area would have required that someone with access to a rabies vaccine would also have access to Plum Brook. This possibility is highly unlikely because access to the base is controlled and I would have encountered some evidence of their activities over the 2 years of my study.

Some proportion of a post-rabies epizootic raccoon population is known to develop RVNA (Carey and McClean 1983). Sero-prevalence of RVNA in a raccoon rabies endemic area has been reported to be 10-28% (Bigler et al. 1973, Jenkins et al. 1988), considerably lower than the 41% I found. This would suggest that the presence of RVNA in a wild raccoon population should be somewhat common in endemic areas. The presence of RVNA in healthy animals is attributed to exposure to the rabies virus, wherein the individual develops RVNA that stave off what would otherwise be an eventually fatal infection. Notably, proportions of RVNA positive animals have also been documented in skunks (*Mephitis mephitis*) (21%) and raccoons (5%) outside of their respective enzootic areas (Rosatte and Gunson 1984, Hill et al. 1992). Further, non-fatal exposure to rabies has also been documented in spotted hyenas (*Crocuta crocuta*) in the Serengeti and in an oncilla (*Leopardus tigrinus*) in Bolivia (East et al. 2001, Deem et al. 2004).

I found strong evidence of a seasonal cycle of RVNA sero-prevalence. The sero-prevalence of RVNA which was quite evident in the early months of trapping (May-June) diminished by August in both years of this study. A similar trend was also found for Old Woman Creek, the control site. These results support the possibility of exposure to a non-raccoon strain rabies virus during early spring.

While translocation of raccoons infected with rabies is known to occur, this is not likely responsible for my RVNA positive titers because all of the raccoons captured during this study appeared healthy and no "sick-looking" animals were reported on Plum

Brook. Translocation of an infected individual into a naïve area with high raccoon density would most likely have resulted in an epizootic outbreak. This was the probable start to the mid-Atlantic raccoon rabies epizootic (Dobson 2000).

Another explanation for the presence of RVNA in a wild raccoon population outside of an enzootic area is the non-fatal infection of individuals exposed to a strain of non-raccoon rabies virus. Cases of both skunk and bat (*Chiroptera*) rabies were documented in Erie County in the last 15 and 4 years respectively. Rabies strains are not host specific as Hill et al. (1993) demonstrated with raccoons developing positive RVNA titers after exposure to the skunk strain of rabies. There is also evidence that rabies infection can occur from exposure to air in caves containing rabid bats as well as ingestion of rabies-infected tissues (Constantine 1967, East et al. 2001).

Raccoons prefer more wooded habitats for availability of den trees, whereas skunks tend to prefer open habitats dominated by grass (Broadfoot et al. 2001). The relatively low number of skunks encountered (only 3 over ~4,000 trap nights) during trapping for both years reduces the probability of a raccoon being exposed to skunk strain rabies virus. Also no cases of skunk rabies have been documented in Erie County since 1989 (ODH 2005).

Bats are the most frequently documented rabies positive taxon of animals in Ohio (Ohio Department of Natural Resources 2003). Furthermore, the big brown bat (*Eptesicus fuscus*), the most common bat in Ohio, is the most frequently confirmed rabies positive species. Although little is known regarding bat populations on PBS, I suggest that the presence of man-made structures on the area would likely provide good roosting sites for bats. While I recognize that exposure of raccoons to the bat strain of rabies may

not completely account for the high RVNA prevalence in the 2003 pre-bait sample, exposure to a non-raccoon strain of rabies also is a plausible explanation of the presence of RVNA outside of an enzootic area.

Effectiveness of Baiting

Thirty-three percent of the target population was RVNA positive (≥0.05 IU/ml) after the first distribution of ORV baits during May 1997 in northeastern Ohio, at an average density of 65 baits/km^2 (ODH 2002). This level of sero-prevalence is not vastly different from what I observed before baiting a naïve area. But without pre-bait serology, measuring the effect of distribution of ORV baits on titer levels and sero-prevalence of RVNA is impossible. In 1998 ODH reported that 32% of samples were RVNA positive after baiting at an average density of 91 baits/km^2. In 1999, after ORV baits were distributed at densities of 75, 150, and 300 baits/km^2, ODH determined the prevalence of RVNA in raccoons in each different bait density area. Sero-prevalence did not differ between the 75 (22%) and 150 (27%) baits/ km^2 but there was an increase to 41% in RVNA sero-prevalence at 300-baits/ km^2 (ODH 2002).

The CDC reports a titer of ≥0.05 IU/ml as positive, and titers of this level have been found to protect an animal against exposure to the rabies virus in laboratory experiments (Rupprecht et al.1988). Pre-bait collection of sera during 2003 indicated that positive RVNA titers (0.05≤-≤0.12 IU/ml) were present in the population. Jenkins et al. (1988) also found RVNA titer levels of 0.05≤-<0.25 IU/ml in raccoon populations and contended that titers <0.25 IU/ml could be attributed to nonspecific antibodies. Therefore, they were thought to be unimportant because they were assumed to be insufficient to protect from infection. However, what titer level is protective against

rabies exposure in a free-ranging raccoon has never been established. Proportions of RVNA positive wild raccoons after distribution of ORV have been determined multiple times since the inception of ORV in the USA, but what constitutes a protective titer has yet to be determined.

A vaccination rate of 63% was found to be sufficient to stop the spread of rabies on a peninsula (Robbins et al. 1998). This is well above even the highest level of ingestion of baits in my population. If all 27% of the population that was tetracycline positive in 2004 was also RVNA positive it is still less than 50% of a level of herd immunity shown to be protective. The 2003 tetracycline data shows a lower (17%) proportion positive and PNA proportions were even lower still. Although naturally occurring PNA were present in the population before distribution of ORV, PNA can still be used as an indicator of immune response to the vaccine vector.

Further, only 8% of my population had positive RVNA titers and of that, only 4%, during both years, exhibited RVNA titers $\geq$0.25 IU/ml after distribution of ORV baits. An average of 22% of the population ingested baits after the bait drops, but only 18% of those animals actually developed high RVNA titers. Data from the vaccine viability analysis indicated that that the baits were not defective. So any animal that ingested the vaccine could have developed RVNA.

I suggest the most likely reason for the difference between my results and those of ODH (2002) is that the population density on Plum Brook was greater than the baited population in eastern Ohio. The ODH trapping area was much larger (~4,000 km^2) and spanned a wider range of habitats (agricultural-urban). Plum Brook Station is relatively small (22 km^2) and the habitat inside the base did not approximate the range seen in the

Ohio operational ORV distribution. The density on Plum Brook is similar to raccoon densities in suburban/urban areas (Prange et al. 2003), like metroparks where a managed habitat is surrounded by an abundant supply of man-made food sources.

The habitat types on and surrounding Plum Brook create an interface where predominantly native vegetation is bounded on one side by development and on the other side by agriculture, an increasingly common occurrence in the mid-western USA. The wooded areas, buildings and floodplains inside Plum Brook likely provide ample resources such as den sites and water sources. Possible den sites, the protection from humans, provided by controlled human access, and the close proximity to anthropogenic food sources like crops, especially corn, and refuse are features that could contribute to a high density raccoon population.

Given the density of raccoons on PBS, a question remains as to the adequacy of 75 baits/km^2 in protecting the population. Wildlife Services distribute baits at a density of 75 baits/km^2, based on a goal of 5 baits distributed/raccoon and a density estimate of 15 raccoons/km^2 (D. Slate, Wildlife Services, personal communication). My population size for Plum Brook at the time of baiting, using my 2003 model M_{bh} estimate, was 1,548 raccoons (Chapter 2). The number of baits distributed based on the Wildlife Services protocol was 1,544. That resulted in just under 1 bait distributed/raccoon in the population. If I had hand-baited off-time areas at 65 baits/km^2 as per the Wildlife Services protocol, only 86 additional baits would have been distributed. For my high density population, 75 baits/km^2 was 20% of the target number of baits/raccoon, also the approximate proportion of my population indicating bait ingestion (22%). Bait density

would have had to be 350 baits/km^2 in order to meet the 5 baits/raccoon goal based on my population size. This emphasizes that there is no universal bait density that will protect raccoon populations in different habitats with different population densities.

MANAGEMENT IMPLICATIONS

This study demonstrates the presence of RVNA in a presumed naïve population and the possibility of underbaiting high density populations. Background serologic surveys should be completed for an area before the distribution of ORV baits. This would allow an accurate measurement of the effect of ORV on the proportion of RVNA positive animals. Pre-bait sampling would also allow for the determination of a background titer level. There also is a critical need for determination of what RVNA titer level is protective for wild raccoons. Without this knowledge there is no directly relevant, tested reference for what proportion of RVNA positive animals in a population could actually survive exposure to rabies.

My results also suggest that for areas with high raccoon densities, as are common in many managed parks across the USA, using a standard baiting density of 75 baits/km^2 is insufficient to protect the population against rabies. Estimation of target population size and correcting bait density accordingly, before the application of ORV baits could not only prove beneficial in controlling the spread of raccoon rabies but could also prove financially beneficial in populations of lower densities by decreasing costs associated with over-baiting.

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	Pre-bait		Post-bait		Total	
	n	%	*n*	%	*n*	%
2003 RVNA Titer (IU/ml)						
≥0.25	0	0	4	4	4	3
0.12<-<0.25	0	0	0	0	0	0
0.05≤-≤0.12	15	41	4	4	19	14
<0.05	22	59	88	92	110	83
Total	37	100	96	100	133	100
2003 PNA Titer (IU/ml)						
≥0.25	0	0	5	5	5	4
0.12<-<0.25	9	24	14	15	23	17
0.05≤-≤0.12	0	0	0	0	0	0
<0.05	28	76	77	80	105	79
Total	37	100	96	100	133	100
2004 RVNA Titer (IU/ml)						
≥0.25	28	9	4	4	32	8
0.12<-<0.25	3	1	1	1	4	1
0.05≤-≤0.12	34	11	4	4	38	9
<0.05	250	79	78	91	328	82
Total	315	100	87	100	402	100

Table 3.1.

Table 3.1. Prevalence of rabies virus-neutralizing antibody (RVNA) and poxvirus-neutralizing antibody (PNA) by titer, for live-trapped raccoons before and after distribution of oral rabies vaccines on Plum Brook Station, Ohio May-October 2003 and March-October 2004.

	RVNA											
	Pre-bait						Post-bait					
	Positive		Negative		Total		Positive		Negative		Total	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
PNA												
Positive	4	27	5	23	9	24	3	38	16	17	19	19
Negative	11	73	17	77	28	76	5	62	77	83	82	81
Total	15	100	22	100	37	100	8	100	93	100	101	100

Table 3.2. Cross tabulations of the prevalence of rabies virus-neutralizing antibody (RVNA) and poxvirus-neutralizing antibody (PNA) for sera collected from live-trapped raccoons before and after the distribution of oral rabies vaccines on Plum Brook Station, Ohio April-October 2003.

	2003 Pre-bait		2003 Post-bait		2004 Pre-bait		2004 Post-bait	
	n	%	*n*	%	*n*	%	*n*	%
Year of Deposition								
2002[a]	0	0	1	1	14	8	1	1
2003	0	0	11	17	93	58	20	26
2004	0	0	0	0	11	7	21	27
No deposition	19	100	53	82	43	27	36	46
Total	19	100	65	100	161	100	78	100

[a]Likely resulting from placebo ORV bait study (Blackwell et al. 2004).

Table 3.3. Prevalence of tetracycline by year of deposition for live-trapped raccoons before and after distribution of oral rabies vaccines on Plum Brook Station, Ohio May-October 2003 and March-October 2004.

	RVNA											
	2003						2004					
	Positive		Negative		Total		Positive		Negative		Total	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Tetracycline												
Positive	2	25	9	10	11	11	3	33	17	22	20	23
Negative	6	75	79	90	85	89	6	67	61	78	67	77
Total	8	100	88	100	96	100	9	100	78	100	87	100

Table 3.4. Cross tabulations of the prevalence of rabies virus-neutralizing antibody (RVNA) and tetracycline for live-trapped raccoons after the distribution of oral rabies vaccines on Plum Brook Station, Ohio September-October 2003 and 2004.

Period	Pre-bait		Pre to post-bait[a]		Post-bait		Post to pre-bait[b]		Total	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
Change										
Increase	1	20	3	37	0	0	2	67	6	35
None	0	0	0	0	0	0	1	33	1	5
Decrease	4	80	5	63	1	100	0	0	10	60
Total	5	100	8	100	1	100	3	100	17	100

[a]Within a single year.
[b]Between 2003 and 2004.

Table 3.5. Change in RVNA titers (IU/ml) of multiple sera samples collected from individual live-trapped raccoons on Plum Brook Station, Ohio May-October 2003 and March-October 2004.

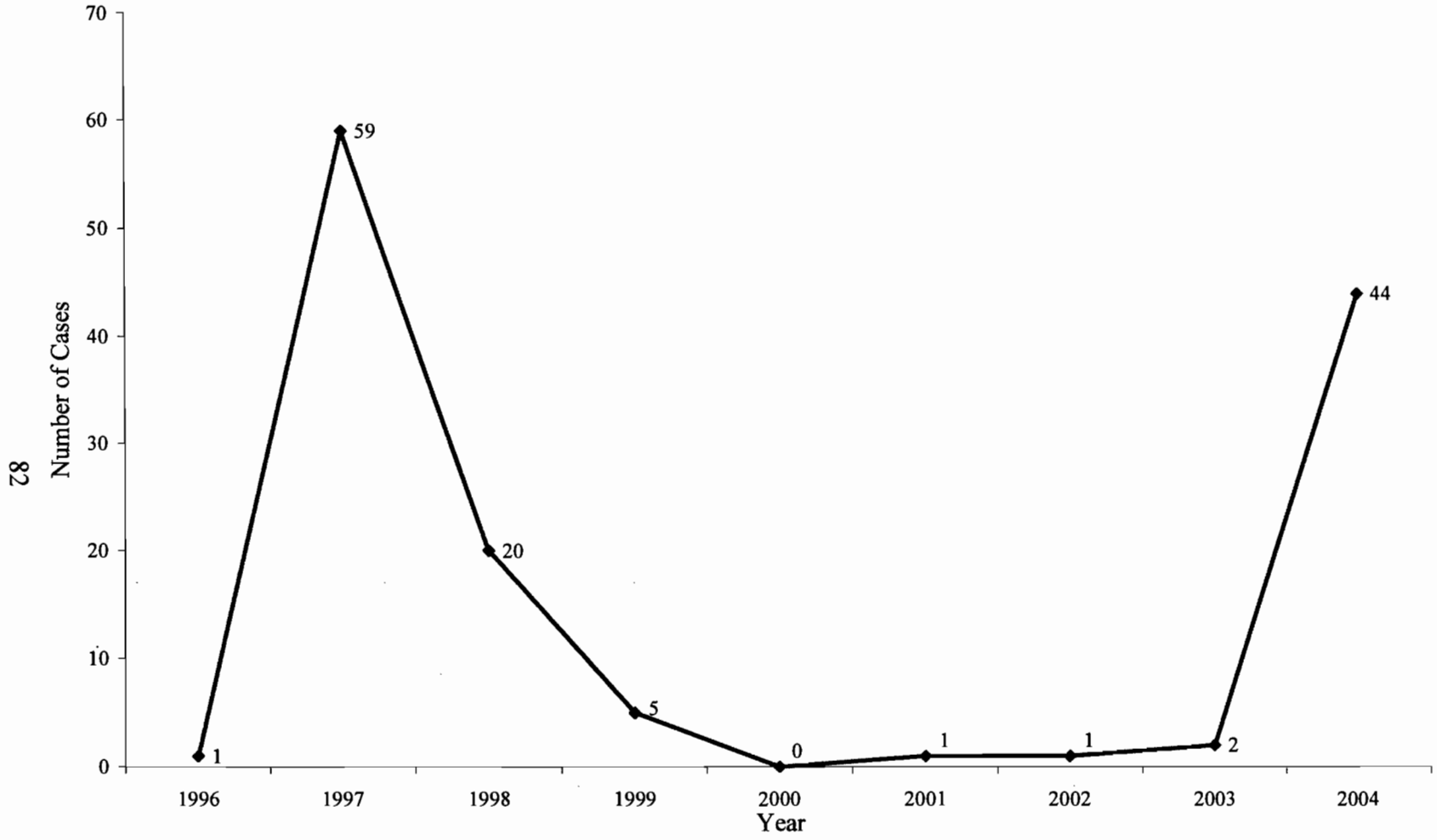

Figure 3.1. Number of reported raccoon rabies cases in Ohio during 1996-2004.

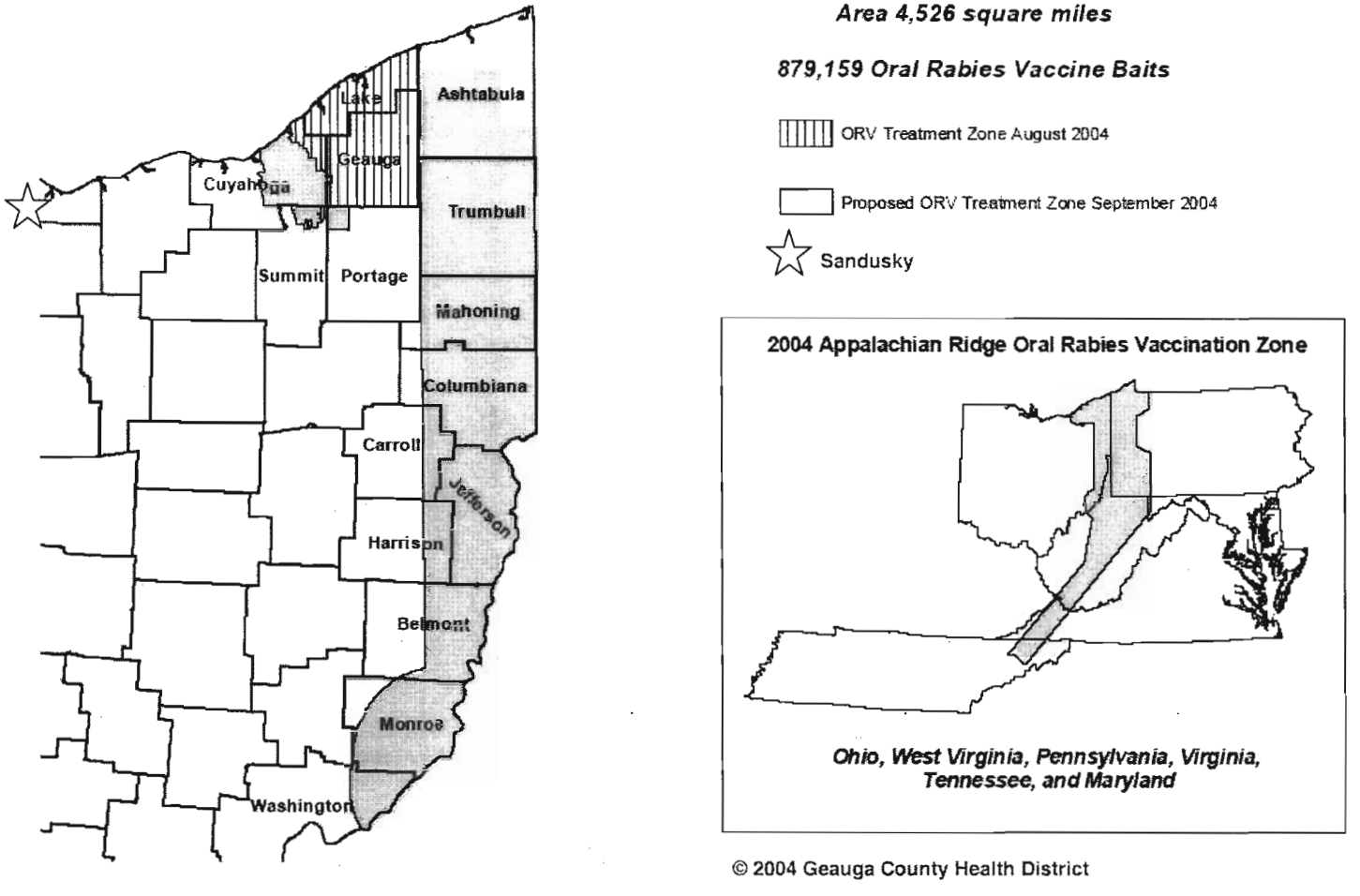

Figure 3.2. Map of the 2004 eastern Ohio Oral Rabies Vaccination (ORV) zone including area, number of baits distributed and relative position to Sandusky and the Appalachian Ridge ORV zone.

Figure 3.3. Map of 22-km^2 Plum Brook Station (PBS), Ohio with 1-km^2 trapping grids and flight lines spaced at 500m used for distribution of ORV during 2003 and 2004.

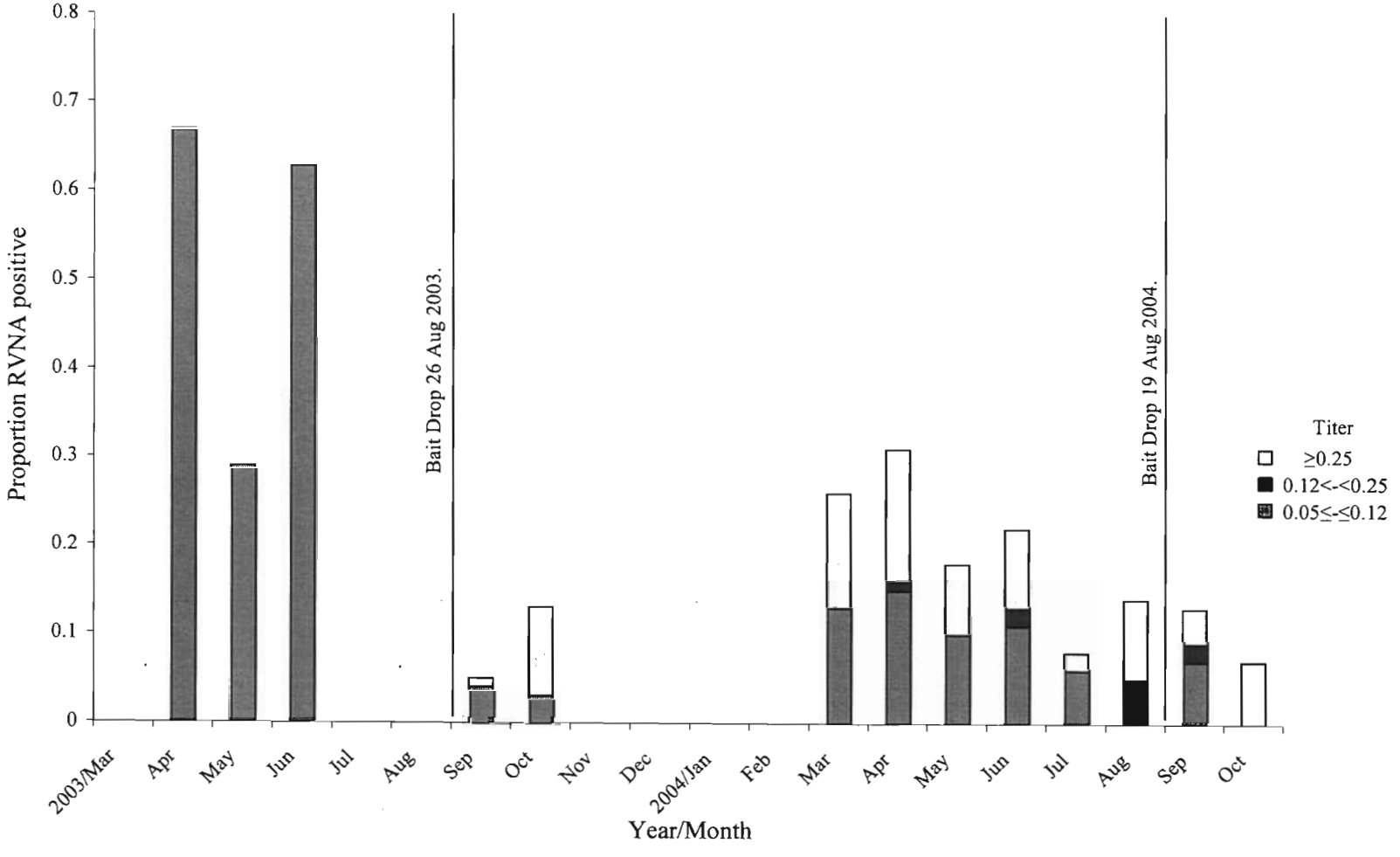

Figure 3.4. Proportion RVNA positive by titer on time for live-trapped raccoons before and after distribution of oral rabies vaccines on Plum Brook Station, Ohio May-October 2003 and March-October 2004.

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