OCCUPANCY, DISTRIBUTION, AND DENSITY OF CARNIVORES WITHIN THE CHERNOBYL EXCLUSION ZONE

by

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(Under the Direction of James C. Beasley and Robert J Warren)

ABSTRACT

All species are exposed to ionizing radiation from both natural and anthropogenic sources. Although it is widely recognized that acute radiation exposure can have negative effects on wildlife, there are significant data gaps regarding the effects of chronic low-dose exposure and no consensus on the potential environmental impacts of nuclear energy or accidents. To elucidate effects of chronic radiation exposure on wildlife, I used multiple non-invasive survey techniques to estimate occupancy, distribution, and density of several species within the Chernobyl Exclusion Zone. I found that radiation density did not significantly influence the aforementioned population characteristics but rather habitat characteristics influenced trends in occupancy and distribution. Furthermore, I found that several mammalian species, including predators such as gray wolves, were abundant throughout the exclusion zone, including areas highly contaminated with radiation. Overall, my results demonstrate that chronic radiation exposure is not limiting the persistence of wildlife species within the Chernobyl Exclusion Zone.

**Key words:** Density, occupancy, radiation, Chernobyl, carnivores, gray wolf

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# CHAPTER 1

## INTRODUCTION AND LITERATURE REVIEW

All species are exposed to ionizing radiation from both natural and anthropogenic sources (Copplestone *et al.* 2001). It is widely recognized that acute radiation exposure can have negative effects on wildlife (Woodwell 1967), and these effects can be lethal or have sub-lethal effects depending on several factors, including dose. However, the most common routes of exposure to domestic and wild animals are chronic and low dose, such as exposure resulting from medical uses, fallout from nuclear bomb testing, emission from nuclear power plants, and/or nuclear accidents (Real *et al.* 2011). Yet, there are significant data gaps regarding the effects of chronic low-dose exposure on wildlife and thus no consensus on the potential environmental impacts of nuclear energy and/or accidents (Hinton *et al.* 2004, 2013). Chronic radiation exposure has the potential to impact species not only at the level of individuals, but also populations if a large proportion of the population accumulates high levels of radiation contamination. However, the effects of chronic radiation exposure on carnivore population dynamics, occupancy, and distribution have not been well studied and are largely unknown.

Chernobyl, and the surrounding human exclusion zone, is an ideal model system to investigate the effects of chronic radiation exposure and lack of anthropogenic pressures on wildlife populations. The Chernobyl Power Plant exploded in 1986. It is located in what is now Pripyat, Ukraine (formally the United Soviet Socialist Republic of

Ukraine at the time of the accident), about 15km south of the current Belarus border. The explosion released around 14 EBq ($14 \times 10^{18}$ Bq) of radioactivity into the atmosphere (World Nuclear Association 2013) and remains the largest single anthropogenic release of radiation into the environment in history, with at least 28 human lives lost due to acute radiation exposure. Additionally, both wildlife and livestock deaths due to acute radiation exposure were reported in the area surrounding the reactor during the days following the accident (Smith and Beresford 2005). Following the accident a human evacuation zone, designated the Chernobyl Exclusion Zone (CEZ), was created surrounding the reactor and covering ~4,300 $km^2$ that spanned across Ukraine and Belarus borders to protect the public from radiological contamination (World Nuclear Association 2013). All citizens within the CEZ were forced to relocate to outside the zone, leaving the landscape within the zone largely uninhabited by humans for now three decades. Throughout the evacuation process and in the years since, no measures have been taken to exclude wildlife from persisting within the CEZ. Thus, several species of wildlife, including many carnivores, exist within the CEZ with little to no human manipulation (International Atomic Energy Agency 2006; Shkvyria and Vishnevskiy 2012).

In the immediate aftermath of the Chernobyl accident, radioactive material that had been suspended in the air by the explosion began to "fall out" and settle onto the landscape haphazardly and indiscriminately, depending upon local wind and rainfall patterns. The highest concentrations settled immediately around the reactor in what is now Ukraine and Belarus (Smith & Beresford 2005). Thus, radionuclides are distributed heterogeneously throughout the landscape surrounding Chernobyl and contamination exposure may be highly dependent on an individual's location, activity, and behavior within that landscape (International Atomic Energy Agency 2006). Although some radionuclide isotopes with short half-lives have decayed to background levels in the CEZ in the 30 years since the accident, others with longer half-lives (e.g., Cesium-137, Strontium-90, Ruthenium-106 and Americium-247) will remain in the ecosystem for 1,000 years or more (Smith and Beresford 2005). These radionuclides have become concentrated within the soil where a substantial proportion remains to this day (Andersson and Roed 1994). Some vertical migration of these contaminants through the soil profile has occurred, although many radionuclides (e.g., $^{137}$Cs) remain available for uptake by biota (Kovalchuk *et al*. 1998; Smith and Beresford 2005). Thus, studies assessing the uptake and effects of radiation on flora and fauna have been an active area of research over the last few decades.

Flora, such as fungi and fruiting plants uptake considerable amounts of $^{137}$Cs and studies have demonstrated negative health impacts in these species from radiation exposure and uptake, creating the possibility of absorption and biomagnification of contaminants in wildlife at higher trophic levels as they move up through the food web (Zhdanova *et al*. 2000; Kovalchuk *et al*. 2003). Indeed, several studies have demonstrated that wildlife within the CEZ accumulate substantial amounts of radiation contamination within their body tissues (Chesser *et al.* 2001; Geras'kin *et al.* 2008; Ryabokon and Goncharova 2006; Yablokov 2009). For example, wild boar, roe deer, and red deer (*Sus scrofa, Capreolus capreolus,* and *Cervus elaphus* respectively) have been shown to uptake substantial amounts of $^{137}$Cs both within the CEZ and other regions of Europe that accumulated a spatially heterogeneous distribution of $^{137}Cs$ contamination following the Chernobyl accident, and absorption varies both spatially and temporally depending upon individual diet, habitat use, and season (Vilic *et al*. 2005; Strebl and Tataruch 2007; Kapala *et al*. 2015).

Some studies conducted within the CEZ, have found evidence of negative health impacts on wildlife including increased rates of DNA mutation, sperm deformities, increased oxidative stress, and increased morbidity and mortality on a population level, especially for birds (Moller *et al*. 2005; Bonisoli-Alquati *et al.* 2010; Moller and Moussaeu 2011; respectively). These findings have led some researchers to conclude that the CEZ represents one of the world's largest ecological sinks because of the high radiation levels that will continue to persist in these environments for centuries or more. However, these studies are limited and controversial because the field methods, analyses, and methods of estimating dose have been questioned (Smith 2008; Beresford and Copplestone 2011). Additionally, recent evidence suggests populations of several species, including large mammals have increased since the accident and continue to thrive within the CEZ due to lack of human manipulation or persecution (Dunin, Pareyko, and Odintsova 1998; Shkvyria and Vishnevskiy 2012; Deryabina *et al*. 2015). Thus, it is still unclear how organisms occupying high trophic levels within the CEZ may be impacted by chronic radiation exposure and inadvertent protection from human persecution; this remains an area of much needed research.

Specifically, there are significant knowledge gaps pertaining to occupancy, distribution, health, and abundance of carnivore species in the radioactively contaminated areas surrounding Chernobyl. These species, including raccoon dog (*Nyctereutes procyonoides*), European badger (*Meles meles*), Eurasian lynx (*Lynx lynx*), gray wolf (*Canis lupus*), brown bear (*Ursus arctos*), and red fox (*Vulpes vulpes*), amongst others, are chronically exposed to radiation of varying amounts, with the level of exposure influenced by diet, behavior, and distribution relative to the distribution of contaminants (Shkvyria and Vishnevskiy 2012). Additionally, in some cases species of international conservation, European bison (*Bison bonasus*) and Przewalski's horse (*Equus ferus przewalskii*), were intentionally introduced into the zone . Others such as Eurasian lynx and brown bear have naturally recolonized the CEZ in the absence of humans. It is unclear what effects, if any, chronic radiation exposure is having on the population characteristics of these species now existing within the CEZ and thus no consensus exists on whether the landscape should be considered a *de facto* nature preserve for these and other threatened, endangered, and vulnerable species in the region or whether targeted management should occur to exclude individuals from highly contaminated areas.

Predators, in particular may be vulnerable to radiation exposure given their trophic position and potential for contaminants to bioaccumulate within food webs (Fuglei *et al*. 2007; Verreault *et al.* 2008). However, to date, few published studies have quantified the effects of radiation on abundance of carnivores and other large mammals within the CEZ. Møller and Mousseau (2013) reported decreased relative abundance of several predator species in areas of higher radiation density in the CEZ, although their study was limited in spatial and temporal extent. In contrast, recent more-extensive studies have found that several mammalian species, including gray wolves, are not only present in areas of high radiation density within the CEZ (Deryabina *et al*. 2015), but populations have increased substantially since the accident (Dunin, Pareyko, and Odintsova 1998; Kuchmel 2006; Deryabina *et al*. 2015). In particular, gray wolves are now widespread throughout the CEZ and populations appear to greatly exceed those observed in other nature reserves in the region (Deryabina *et al*. 2015; Webster *et al*. *In Press*). Although these studies suggest that the abundance of many wildlife species have increased in the zone since the evacuation of humans, they have relied on indices of abundance rather than explicit measures of animal density, especially for large mammals. As such, in the absence of quantitative measures of animal density, especially for large mammals such as wolves, there remains a vigorous scientific and public debate regarding the impacts of chronic radiation exposure on wildlife populations. Thus, further study is vitally needed to better elucidate how chronic radiation exposure affects populations of free-ranging carnivores and other mammals within the CEZ.

Other areas abandoned by humans, for example the demilitarized zone between North and South Korea and tracts of former agricultural lands in Europe and the U.S., benefit from lack of human settlement (Kim 1997; Bowen *et al*. 2007; Navarro and Pereira 2012;). Although wildlife within the CEZ are generally protected from human population pressures, it is important to consider the effects of chronic radiation exposure on wildlife to allow managers and policy makers to effectively manage wildlife populations in the CEZ and other areas with known contamination releases. If the negative health impacts on wildlife are severe at a population level (i.e., diminished genetic diversity, fecundity, or survival of all age classes), then the landscape could be acting as an ecological trap for the several wildlife species known to be present within the CEZ. However, in the absence of population-level effects, such landscapes may serve as important refugia to wildlife given the absence of other human pressures. Additionally, such data will be informative in guiding long-term management decisions in nations where more recent nuclear accidents have occurred such as the reactor accident in Fukushima, Japan (Yasnuri *et al*. 2011). Moreover, Ukraine recently announced its intention to designate the portion of CEZ that falls within its borders as a nature preserve in the coming months and the nation currently allows a small tourism industry to flourish by allowing visitors into the CEZ on a regular basis (Anisimov and Ryzhenkov 2014). By understanding long-term effects of radiation contamination on wildlife in the CEZ, researchers and policy makers will better be able to effectively protect and manage these designated "disaster areas" for long-term recovery and conservation.

My thesis research investigating how occupancy, distribution, and density of mammalian species within the CEZ are influenced by radiation exposure will help elucidate the long-term effects of chronic radiation exposure on the population characteristics of mammalian species, especially carnivores such as gray wolves. This critically needed research will allow researchers and managers to more effectively manage other contaminated or abandoned landscapes to benefit wildlife species. In Chapter 1 of my thesis, I present a literature review of this area of research. In Chapter 2, I use remote cameras to quantify the influence of radiation on occupancy and distribution of four mammalian species, red fox, raccoon dog (*Nyctereutes procyonoides*), Eurasian boar (*Sus scrofa*), and gray wolves (*Canis lupus*), within the CEZ. This is an essential first step in elucidating how wildlife are distributed across areas of spatially heterogeneous radiation contamination within the CEZ. In Chapter 3, I expand upon the work in Chapter 2 to estimate density of an apex predator, gray wolves, across the Polesie State Radioecological Reserve (PSRER) and relate areas of high wolf density to environmental attributes such as contaminant density and land cover type. This research will provide the first robust estimates of density for gray wolves within the CEZ and contribute to our scientific understanding of how radiation contamination density may be influencing the population dynamics of an apex predator. Overall, this research presents critical progress towards understanding the impact of chronic radiation exposure on wildlife species within the CEZ.

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# CHAPTER 2

# WHERE THE WILD THINGS ARE: INFLUENCE OF RADIATION ON THE DISTRIBUTION OF FOUR MAMMALIAN SPECIES WITHIN THE CHERNOBYL EXCLUSION ZONE

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## Abstract

Although nearly 30 years have passed since the Chernobyl Nuclear Power Plant accident near the town of Pripyat, Ukraine, the status and health of mammal populations within the Chernobyl Exclusion Zone (CEZ) remain largely unknown, and are of substantial scientific and public interest. Information regarding the response of flora and fauna to chronic radiation exposure is important in helping us understand the ecological consequences of past (eg Chernobyl and Fukushima) and potential future nuclear accidents. We present the results of the first remote-camera scent-station survey conducted within the CEZ. We observed individuals of 14 mammalian species in total; for those species with sufficiently robust visitation rates to allow occupancy to be modeled (gray wolf [*Canis lupus*], raccoon dog [*Nyctereutes procyonoides*], Eurasian boar [*Sus scrofa*], and red fox [*Vulpes vulpes*]), we found no evidence to suggest that their distributions were suppressed in highly contaminated areas within the CEZ. These data support the results of other recent studies, and contrast with research suggesting that wildlife populations are depleted within the CEZ.

## Introduction

There are 438 operational nuclear reactors worldwide, and nuclear power production is expected to grow over the coming decades (IAEA 2014). As this industry expands, so too does the need to fully understand the ecological consequences of its production and associated accidents. Although nuclear energy production has strict protocols to protect human health and maximize safety, the potential for a nuclear accident capable of causing catastrophic, ecosystem-level radiation contamination (eg Chernobyl and Fukushima) is always present. The consequences of such accidents are costly from an economic, human health, and ecologcial perspective, and can render affected regions uninhabitable to humans for centuries. Thus, the potential long-term ecological effects of nuclear disasters are of global interest to international organizations and the public.

Chernobyl is a prime location for investigating such effects on wildlife populations. The 1986 accident at the Chernobyl Nuclear Power Plant resulted in the release of large amounts of radioactivity into the atmosphere. In response, humans were evacuated from a roughly 4300-km$^2$ area spanning the modern borders of Ukraine and Belarus, an area now referred to as the "Chernobyl Exclusion Zone" (CEZ). Following the accident, radioactive material suspended in the air began to "fall out" and settle onto the landscape, depending upon local wind and rainfall patterns (Smith and Beresford 2005). Radionuclides were deposited in a spatially heterogeneous manner across the landscape surrounding Chernobyl, and contaminant exposure rates in wildlife species are therefore highly dependent on an individual animal's diet, behavior, and location within that landscape. Despite this contamination, many wildlife species have continued to inhabit the CEZ with little to no human manipulation; in some cases, wildlife were intentionally introduced into the zone by humans (ie European bison [*Bison bonasus*] and Przewalski's horse [*Equus ferus przewalskii*]; Shkvyria and Vishnevskiy 2012).

During the nearly 30 years since the accident, numerous studies have been conducted within the CEZ to determine the impact of radiation on the region's flora and fauna. Several studies have shown that wildlife within the zone continue to accumulate substantial amounts of radionuclides (Chesser *et al*. 2001; Geras'kin *et al*. 2008); other research has uncovered a range of negative health impacts on wildlife, including sperm deformities and increases in mutation rates, morbidity, and mortality (Møller *et al*. 2005). However, controversy has surrounded some studies that documented severe health effects in wildlife, due to questions regarding field methods, analyses, and methods of estimating radiation doses (Beresford and Copplestone 2011). Furthermore, few studies have examined organisms occupying high trophic levels within the Chernobyl ecosystem, and so little is known about how chronic radiation exposure may be affecting mid- to large-sized mammals.

Specifically, there are large knowledge gaps pertaining to occupancy trends and densities of mammals in the CEZ, and it is unclear what effects chronic radiation exposure may have on their population status and distribution; such data are vital for informing future management or protection of wildlife inhabiting contaminated landscapes. Recent evidence suggests that populations of several large mammal species increased within the CEZ during the first decade after the accident, and that large mammal distributions are uncorrelated with severity of radiation contamination (Deryabina *et al*. 2015), in contrast to previous findings from a more limited spatial and temporal study (Møller and Mousseau 2013). Thus, additional research that more clearly records wildlife distribution, abundance, and health is needed. The goal of our study was, for the first time, to use scent stations coupled with remote cameras to determine whether the probability of mammal occurrence is correlated to the intensity of radionuclide contamination within the CEZ.

## Methods

### *Study area*

We conducted our study in the 2162-$km^2$ Polesie State Radioecological Reserve (PSRER) in southern Belarus. The reserve was created in 1988 by the regional government to encompass the portion of the CEZ located within the Belarusian Soviet Socialist Republic (which at that time was a component of the Soviet Union but is now independent Belarus). Approximately 51% of the PSRER is forested, with the remaining 49% composed of abandoned agricultural and developed land (deserted villages, farms, and transportation systems), open fields, and seasonal wetlands. The PSRER is bisected by the Pripyat River, and human access to the reserve is strictly regulated. Levels of cesium-137 ($^{137}Cs$) within the PSRER remain very high, and as of 2009 soil contaminant densities ranged from 40 kiloBecquerels per square meter (kBq $m^2$) to >7500 kBq $m^2$ across the reserve (Figure 1; Izrael and Bogdevich 2009). Because radionuclides are unevenly distributed across the landscape, exposure rates will differ widely among wildlife species.

### *Field sampling*

We deployed scent stations throughout the PSRER from October–November 2014. Scent stations consisted of a plaster tab infused with fatty acid scent (US Department of Agriculture, Pocatello, ID) placed in a 0.9-m-diameter circle of soil cleared of vegetation.

Stations were sited a minimum of ~3 km apart, to reduce the chances of individuals visiting multiple stations within a single survey period. In addition, we placed all stations <10 m from a road, given that many carnivores use roads as a means of travel (Macdonald 1980). We affixed an infrared remote camera (Moultrie m990i Infrared Game Camera, EBSCO Industries Inc) to a tree or other vertical structure within 3 m of each station. Cameras were programmed to take three pictures each time they were triggered by nearby movement, with a 5-second delay between events. Stations were active for 7 days, and were revisited on day 3 or 4 to replace scent tabs if tampered with, to minimize variation in detection probability throughout the sampling period.

*Quantifying habitat*

Using GPS technology, we demarcated 42 sites located in various habitat types to obtain representative data for each habitat type that occurs within the reserve. GPS-based positions were used in a supervised classification (ArcGIS 10.2.1, ESRI, Redlands, CA) of LANDSAT imagery obtained from GLOVIS (US Geological Survey Global Visualization Viewer, http://glovis.usgs.gov). We reclassified the content of the resulting map into five habitat types: pine forest, hardwood [deciduous] forest, seasonal marsh, dry field, and water. Anthropogenic structures (eg roads, houses, public buildings) were not considered barriers to movement or occupancy because of the lack of continuous human presence within the PSRER over the past 30 years. We used the Geospatial Modelling Environment (Spatial Ecology LLC, www.spatialecology.com/gme) platform to quantify habitat cover within circular buffers with radii of 250 m and 1000 m at each sampling location; these distances were chosen in order to capture both fine and landscape-level scales of potential habitat selection for our species of interest. Within each circular buffer, we calculated area-weighted mean soil activity densities of $^{137}$Cs (“Rad”; kBq m$^{-2}$; based on geo-rectified imagery data from Izrael and Bogdevich 2009), as well as area of forest (“Forest”), area of open field ("DryField"), area of seasonal wetland (“Marsh”), quantity of edge habitat (length, in meters, of intersection of open and forested habitats; “Edge”), distance of sampling location to the Pripyat River (“Water”), and distance of sampling location to the CEZ border as an indicator of sensitivity to anthropogenic pressures (“Border”).

*Data analysis*

On the basis of average home-range sizes of carnivores detected in our camera surveys, we considered each station as an independent sample for each species of interest, excluding gray wolves, which have average home-range sizes of 600 km$^2$ to 900 km$^2$ in the region (Theuerkauf *et al*. 2003). We divided the survey period into seven 24-hour sampling occasions during deployment of scent stations and used these data to create species-specific binary detection histories for each station. We used an occupancy modeling approach to quantify the influence of radiation and habitat attributes on the distribution of surveyed wildlife throughout the PSRER. This approach (McKenzie *et al*. 2002) relies on detection/non-detection data and maximum likelihood estimation to quantify the probability of site occupancy (ie probability of a given animal being present at a site during the period of sampling, denoted as $\Psi$) and detection probability (probability of detecting a species at an occupied site) while incorporating our detection/non-detection data as well as covariates of interest. We used a single-season occupancy analysis and developed models only for species that met a minimum threshold of 10 detections (the number of visitations to stations required for modeling to be successful). We did not model temporal variation in detection probabilities as a function of survey-specific variables (eg weather events), but rather we made the assumption that, based on our sampling design, no survey-specific variables influenced detection probability throughout the 7-night survey period.

To avoid multicollinearity, we examined correlations among the environmental variables at both spatial scales (250 m and 1000 m) by deriving a matrix of all possible Pearson correlation coefficient values. Any variables with a significant correlation ($r^2 \geq 0.2$; $P \leq 0.05$) were not simultaneously included in the same model in subsequent analyses. Preliminary analyses revealed models containing ≥3 uncorrelated variables failed to converge for every species; we therefore limited our analyses to models including ≤2 environmental variables. We developed a suite of 15 candidate models at each habitat scale that incorporated all combinations of uncorrelated variables and used them for every species.

We conducted all analyses using R (R Development Core Team, www.r-project.org) and fitted the model using the package "unmarked" (Fiske and Chandler 2011), which accounts for potential autocorrelation of data when calculating both detection probabilities and site occupancy probability, but assumes spatial independence between survey locations. We calculated Akaike Information Criterion (AIC) values for all models, and ranked models based on ΔAIC and AIC weights ($w_i$) to determine which model best fit the capture history data (Burnham and Anderson 2002). We used the chi-square ($\chi^2$) method for site-occupancy models to ensure model validity (MacKenzie and Bailey 2004). We averaged all models within two AIC units and derived parameter beta estimates from the averaged model. Uninformative parameters were identified by calculating 85% confidence intervals (CIs) for model-averaged parameter estimates for each species and scale (Arnold 2010).

## Results

We deployed 98 scent stations over a 5-week period in October–November 2014. Of these stations, four were excluded due to camera malfunctions, making our effective sample size 94. We detected 14 mammal species (including seven carnivores) at scent stations, four of which – gray wolf (*Canis lupus*), raccoon dog (*Nyctereutes procyonoides*), red fox (*Vulpes vulpes*), and Eurasian boar (*Sus scrofa*) – had sufficient detection thresholds to successfully model occupancy (Table 1; Figure 2; WebFigure 1). Although carnivores were the main focus of this study, visitation rates by the omnivorous Eurasian boar (an artiodactyl species) were adequately robust to model visitation and therefore this species was included in the analyses. Supported models varied among species across both spatial scales, although several models contained uninformative parameters that were removed from biological interpretation (Table 3).

Radiation did not negatively affect occupancy probability ($\Psi$) for any species or spatial scale examined. For red foxes, all variables within supported models were uninformative at the 250-m scale. At the 1000-m scale, “Water” ($\beta = 4.02$, CI = 0.38 – 7.65) and “Marsh” ($\beta = -2.07$, CI = –4.05 – –0.09) had a positive and negative influence on $\Psi$, respectively (Table 2). For Eurasian boars, distance to the CEZ border negatively influenced $\Psi$ at both habitat scales ($\beta = -2.21$, CI = –3.64 – –0.77; Table 2); all other parameters in supported models were uninformative. For raccoon dogs, “Rad+Water” was the most supported model at both spatial scales, with both of these parameters having a positive influence on Ψ (Table 2). Additionally, at the 1000-m scale, "DryField" negatively influenced Ψ ($\beta = -1.62$, CI = –3.1 – –0.14). For gray wolves, no measured environmental attributes were informative for estimating Ψ, but we anticipated this possibility, given that the model assumption of independence of sampling stations was violated for this species due to their large home-range sizes.

## Discussion

Our results provide the first quantitative analysis on the distribution of carnivores within the CEZ based on remote-camera surveys. The data suggest that the current distribution of wildlife within the CEZ is unaffected by $^{137}Cs$ contaminant densities. However, we did not examine the health effects of radiation exposure at the individual level, and contaminant densities of $^{137}Cs$ may not directly correlate to absorbed dose rate due to a multitude of factors (eg movement, behavior, diet). Long-term, chronic exposure to radiation may possibly affect animal health, although our findings indicate that current levels of exposure are not limiting the distributions of gray wolf, red fox, raccoon dog, or Eurasian boar. Moreover, if individual-level effects were severe, we would expect a negative correlation between occupancy probability and radiation contaminant density, particularly for species with restricted home-range sizes (eg raccoon dog, red fox), a pattern not supported by our data.

Indeed, individuals of all four species included in our analyses were detected at stations <500 m from areas with the highest contaminant densities of $^{137}Cs$ in the PSRER ($\geq$7500 kBq $m^{-2}$). These species included raccoon dogs and red foxes, which have home-range sizes of only 1.5–2.0 $km^2$ (Drygala and Zoller 2012), and are therefore highly influenced by local radiation levels. The occupancy probability for raccoon dogs was positively correlated with radiation contaminant density at both spatial scales measured, as well as with distance to the Pripyat River. A positive correlation with radiation level is unexpected, and most likely due to environmental factors not measured in our study (eg prey base, interspecific competition). Similarly, red foxes were unaffected by $^{137}Cs$ contaminant density, and had a higher probability of occupying areas farther from the Pripyat River and areas with less seasonal marsh, consistent with habitat requirements for this species.

Eurasian boars also have relatively small home ranges (3–15 $km^2$; Baskin and Danell 2003) and so are likely to be affected by local radiation levels. However, our data do not indicate that populations of Eurasian boars were suppressed in highly contaminated regions of the CEZ, as only distance to the CEZ border was found to influence boar occupancy probability. This correlation, which was negative at both habitat scales, most likely exists because the landscape immediately outside the CEZ is predominantly composed of agricultural croplands. Eurasian boars utilize agricultural crops as a food resource, so areas adjacent to the CEZ border probably support higher densities of boars because they offer increased foraging opportunities. Although this trend coincides with boars being more likely to be found in areas of lower radiation, $^{137}Cs$ contaminant density was not found to influence occupancy, and boars were detected at stations in the most contaminated regions of our study area.

Gray wolves were unique among the species considered in that no measured environmental parameters were found to be influential at either scale. We expected that the effects of $^{137}Cs$ contaminant density on their occupancy probability would be limited because of their large home-range size and high mobility through spatially heterogeneous regions of radiation contamination. Although our data support this hypothesis, we acknowledge that our interpretations are limited for wolves, as our study design was based on the assumption that an individual animal cannot visit multiple stations in a single sampling occasion; this assumption should not hold true for members of this species, given their characteristically extensive home ranges.

Overall, our findings indicate that the severity of radiation contamination has no discernible impact on the current distribution of selected mid- to large-sized carnivores, or of Eurasian boars, within the CEZ. Rather, other habitat-related and anthropogenic factors (eg agricultural lands, human presence) appear to be driving occupancy. Thus, despite severe impacts on some wildlife immediately after the nuclear accident (Alexakhin and Geras'kin 2013), our results corroborate the conclusions of Baker *et al*. (1996) and Deryabina *et al*. (2015), and suggest that robust populations of numerous mammals now occur throughout much of the CEZ, including areas with radiation levels exceeding 7500 kBq $m^{-2}$. Such data contribute to an improved scientific understanding of the long-term ecological consequences of nuclear accidents, and can be applied by policy makers to establish effective management and safety protocols for wildlife in highly contaminated landscapes elsewhere. However, further studies are needed to elucidate whether, and to what extent, critical attributes of wildlife populations (eg abundance, genetic diversity) or individuals (eg genetic mutations, fecundity, survival) are affected by chronic radiation exposure.

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**Table 2.1**. Species detected within the PSRER from scent-station surveys conducted in fall 2014

| Species detected | Number of stations occupied | Total detections** |
|---|---|---|
| Black grouse (*Tetrao tetrix*) | 2 | 4 |
| Domestic dog (*Canis familiaris*) | 2 | 2 |
| Eurasian badger (*Meles meles*) | 9 | 9 |
| Eurasian bison (*Bison bonasus*) | 1 | 1 |
| Eurasian boar (*Sus scrofa*)* | 9 | 21 |
| Eurasian jay (*Garrulus glandarius*) | 10 | 10 |
| Eurasian magpie (*Pica pica*) | 2 | 4 |
| Eurasian red squirrel (*Sciurus vulgaris*) | 2 | 4 |
| European hare (*Lepus europaeus*) | 5 | 5 |
| Gray wolf (*Canis lupus*)* | 15 | 26 |
| Least weasel (*Mustela nivalis*) | 3 | 3 |
| Moose (*Alces alces*) | 3 | 3 |
| Pine marten (*Martes martes*) | 7 | 7 |
| Raccoon dog (*Nyctereutes procyonoides*)* | 31 | 60 |
| Red deer (*Cervus elaphus*) | 2 | 2 |
| Red fox (*Vulpes vulpes*)* | 9 | 10 |
| Roe deer (*Capreolus capreolus*) | 2 | 2 |

**Notes**: *species included in modeling analysis; **detections = number of 24-hour sampling occasions in which species was observed at scent stations.

**Table 2.2**. Model-averaged parameter estimates and 85% confidence intervals derived from scent-station survey data collected across the PSRER during fall 2014.

| *Species* | *Scale* | *Parameter* | *Parameter estimate* | *CI (85%)* |
|---|---|---|---|---|
| Red fox | 250 | Water* | 4.5 | –0.86 – 9.87 |
| | | Rad* | –2.03 | –5.93 – 1.87 |
| | | Edge* | –37.69 | –127.78 – 52.4 |
| | | Forest* | 19.56 | –28.89 – 68.01 |
| Red fox | 1000 | Water | 4.02 | 0.38 – 7.65 |
| | | Marsh | –2.07 | –4.05 – –0.09 |
| Boar | 250 | Border | –2.21 | –3.64 – –0.77 |
| | | Edge* | 0.47 | –0.35 – 1.29 |
| | | Rad* | 0.15 | –0.65 – 0.55 |
| Boar | 1000 | Border | –2.21 | –3.64 – –0.77 |
| | | Edge* | 0.47 | –0.35 – 1.29 |
| | | Rad* | 0.15 | –0.65 – 0.94 |
| Raccoon dog | 250 | Rad | 3.03 | 0.59 – 5.47 |
| | | Water | 4.24 | 1.52 – 6.96 |
| Raccoon dog | 1000 | Rad | 3.71 | 0.56 – 6.85 |
| | | Water | 3.77 | 0.65 – 6.88 |
| | | Edge* | –31.29 | –94.53 – 31.95 |
| | | Border* | –20.66 | –61.28 – 19.96 |
| | | DryField | –1.62 | –3.1 – –0.14 |
| Gray wolf | 250 | Constant | –1.08 | –1.73 – –0.42 |
| | | Edge* | 0.39 | –0.11 – 0.88 |
| | | DryField* | –0.22 | –0.73 – 0.29 |
| | | Marsh* | –0.13 | –0.62 – 0.36 |
| | | Border* | 0.11 | –0.37 – 0.59 |
| | | Water* | –0.03 | –0.51 – 0.45 |
| | | Rad* | 0.02 | –0.44 – 0.47 |
| Gray wolf | 1000 | Constant | –1.08 | –1.73 – –0.42 |
| | | DryField* | 0.2 | –0.28 – 0.67 |
| | | Marsh* | –0.15 | –0.64 – 0.34 |
| | | Edge* | 0.12 | –0.38 – 0.62 |
| | | Border* | 0.11 | –0.37 – 0.59 |
| | | Rad* | 0.04 | –0.4 – 0.49 |
| | | Water* | –0.03 | –0.51– 0.45 |

**Notes**: Only models ranked within two AIC units were averaged to obtain parameter estimates. *Parameter was uninformative and was not included in biological interpretation.

**Table 2.3**. Model rankings for several species derived from scent-station survey data collected across the PSRER during fall 2014 at two habitat scales, 250 m and 1000 m.

| *Species* | *Habitat scale* | *Model name* | *K* | *AIC* | *ΔAIC* | *AICw* |
|---|---|---|---|---|---|---|
| Red fox | 250 | Water | 3 | 103.99 | 0.00 | 0.27 |
| | | Rad+Water | 4 | 104.69 | 0.70 | 0.19 |
| | | Edge+Forest | 4 | 105.08 | 1.09 | 0.15 |
| | | Water+DryField | 4 | 105.11 | 1.12 | 0.15 |
| Red fox | 1000 | Water+Marsh | 4 | 102.18 | 0.00 | 0.40 |
| | | Water | 3 | 103.99 | 1.81 | 0.16 |
| Boar | 250 | Border | 3 | 108.1 | 0.00 | 0.50 |
| | | Edge+Border | 4 | 109.3 | 1.20 | 0.27 |
| | | Rad+Border | 4 | 110.03 | 1.93 | 0.19 |
| Boar | 1000 | Border | 3 | 108.1 | 0.00 | 0.50 |
| | | Edge+Border | 4 | 109.79 | 1.20 | 0.27 |
| | | Rad+Border | 4 | 110.03 | 1.93 | 0.19 |
| Raccoon dog | 250 | Rad+Water | 4 | 286.11 | 0.00 | 0.59 |
| Raccoon dog | 1000 | Rad+Water | 4 | 287.32 | 0.00 | 0.36 |
| | | Edge+Border | 4 | 288.00 | 0.69 | 0.25 |
| | | Water+Field | 4 | 288.54 | 1.23 | 0.19 |
| Gray wolf | 250 | Constant | 2 | 172.67 | 0.00 | 0.19 |
| | | Edge | 3 | 173.34 | 0.66 | 0.14 |
| | | DryField | 3 | 174.27 | 1.60 | 0.09 |
| | | Marsh | 3 | 174.53 | 1.85 | 0.08 |
| | | Border | 3 | 174.56 | 1.89 | 0.07 |
| | | Water | 3 | 174.66 | 1.99 | 0.07 |
| | | Rad | 3 | 174.67 | 2.00 | 0.07 |
| Gray wolf | 1000 | Constant | 2 | 172.67 | 0.00 | 0.22 |
| | | DryField | 3 | 174.31 | 1.64 | 0.10 |
| | | Marsh | 3 | 174.47 | 1.8 | 0.09 |
| | | Edge | 3 | 174.55 | 1.88 | 0.09 |
| | | Border | 3 | 174.56 | 1.89 | 0.09 |
| | | Rad | 3 | 174.65 | 1.98 | 0.08 |
| | | Water | 3 | 174.66 | 1.99 | 0.08 |

**Notes**: K = number of parameters included in model; AICw = assigned weight of model within rankings.

**Figure 2.1.** The Polesie State Radioecological Reserve (PSRER) has considerable heterogeneity in $^{137}Cs$ soil contaminant density, ranging from 40 kBq $m^{-2}$ to >7500 kBq $m^{-2}$, as derived from imagery reported by Izrael and Bogdevich (2009) and imported to ArcGIS 10.2, georectified, and digitized. Green dots represent the locations of the scent stations.

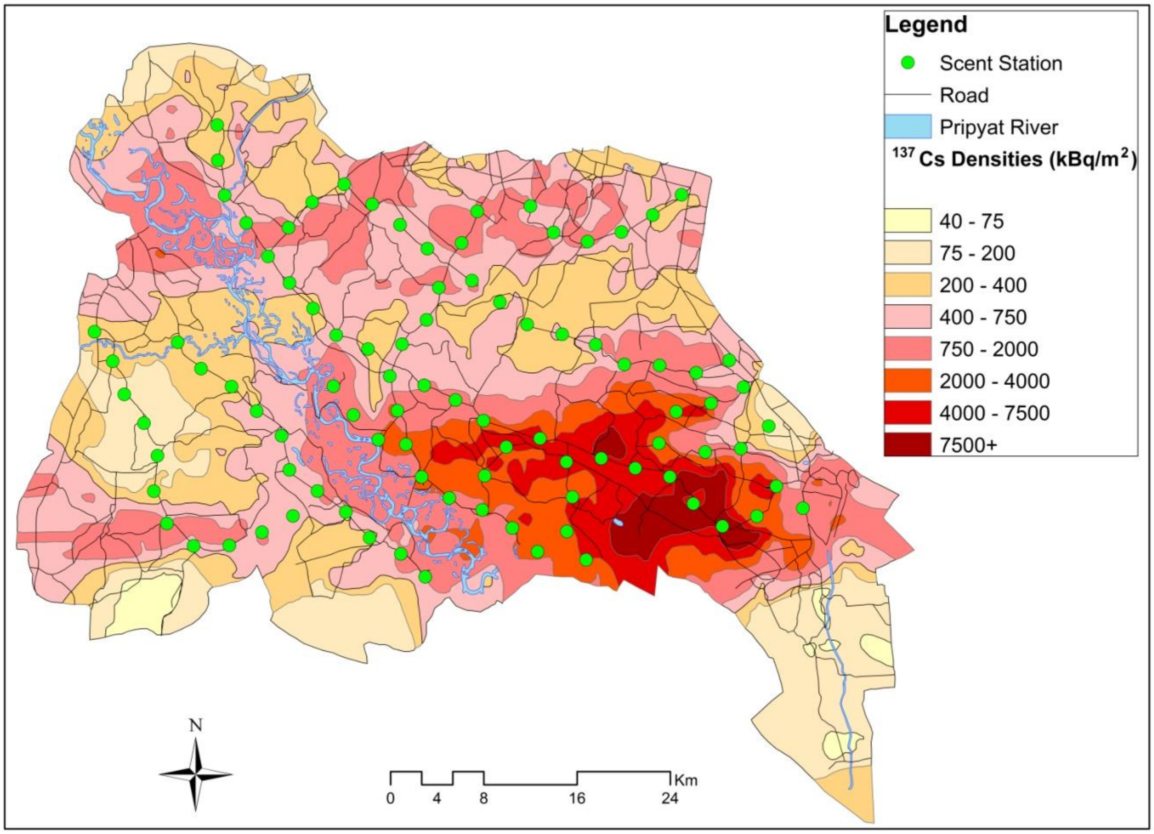

**Figure 2.2.** Photographs of several species of carnivores observed visiting scent stations deployed throughout the PSRER during fall 2014: (a and d) gray wolf (*Canis lupus*), (b) raccoon dog (*Nyctereutes procyonoides*), and (c) red fox (*Vulpes vulpes*).

**Figure 2.3**: Fourteen species of mammals were detected visiting scent stations deployed throughout the PSRER during fall 2014. Species shown here are (a) pine marten (*Martes martes*), (b) European bison (*Bison bonasus*), (c) Eurasian badger (*Meles meles*), and (d) Eurasian boar (*Sus scrofa*).

# CHAPTER 3

# SPATIAL HETEROGENEITY IN DENSITY OF GRAY WOLVES WITHIN THE CHERNOBYL EXCLUSION ZONE

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## Abstract

As the nuclear energy production industry continues to experience worldwide growth, there is an increasing need to understand potential effects of industrial operations and accidents on surrounding landscapes. Knowledge about the response of flora and fauna to chronic radiation exposure is important in helping to understand the ecological consequences of past (e.g., Chernobyl and Fukushima) and potential future nuclear accidents. Here, we present the first use of molecular scatology to estimate the density of gray wolves (*Canis lupus*) across varying levels of $^{137}Cs$ contamination densities within the Chernobyl Exclusion Zone (CEZ). We identified 138 wolf scats collected within the zone to individual, and using a spatially explicit capture-recapture framework to derive an estimated wolf density of 106 /1000 $km^2$. Additionally, we investigated potential associations of wolf density to environmental factors such as $^{137}Cs$ contamination density and dominant habitat type (i.e., pine forest, hardwood forest, seasonal marsh, and dry field). We found that areas of relatively high wolf density were associated with $^{137}Cs$ contamination densities ranging from 75-7,499 $kBq/m^2$, but there was no relationship between habitat type and contamination density. However, areas of high wolf density had significantly higher radiation density than areas of low wolf density. Our findings suggest that wolf density is not diminished in areas of high contamination density when compared to areas of low contamination density or limited to certain habitat types within the CEZ.

## Introduction

The nuclear energy production industry is expected to continue to expand in the coming years as a sustainable energy source (IAEA, 2014). Although the rate of accidents in nuclear energy production is very low, the risk of a nuclear accident resulting in catastrophic radiation contamination of human workers and citizens, as well as the surrounding ecosystem still persists. Thus, in the light of the growing nuclear industry, it is critical to understand the short- and long-term ecological effects of a nuclear accident on the surrounding landscape, including potential effects on wildlife populations. It is well established that acute, high levels of radiation exposure can have severe negative health impacts on wildlife (Woodwell 1967). However, uncertainty remains as to how chronic exposure to sub-lethal radiation contamination may affect the ability of wildlife populations to persist over time.

Chernobyl, and the surrounding landscape represent the ideal model in which to study the long-term effects of ionizing radiation on wildlife populations. The Chernobyl Power Plant exploded in 1986. It is located in what is now Pripyat, Ukraine about 15km south of the current Belarus border. The explosion released around 14 EBq (14 x $10^{18}$ Bq) of radioactivity (e.g., $^{131}$I, $^{137}$Cs, $^{90}$Sr and $^{241}$Pu, which breaks down into $^{241}$Am, a more toxic compound with a longer half-life) into the atmosphere (Kovalchuk *et al*. 1998; Kashaprov *et al.* 2001; Smith and Beresford 2005; World Nuclear Association 2013) and was the largest single anthropogenic release of radiation into the environment in history, with at least 28 human lives lost due to acute radiation exposure. Over the next weeks and months, the airborne contamination settled onto the surrounding landscape depending on local wind and rainfall patterns, creating a spatially heterogeneous distribution of radiation in the environment (Smith and Beresford 2005).

Contamination spread as far north as the Arctic Circle in Norway, as far west as central Russia, and as far south as the Black sea, covering a large portion of Europe in radioactive materials of varying intensities (Alexakhin and Geras'kin 2013, Copplestone 2001). In the 2 years after the accident, the Union of Soviet Socialist Republics (USSR) created a human evacuation zone around the Chernobyl reactor and displaced more than 300,000 people to contain ~4,300 $km^2$ of contaminated landscapes, which is now known as the "Chernobyl Exclusion Zone" (CEZ). However, no measures were taken to exclude wildlife from the region, and in some cases species of international conservation concern, European bison (*Bison bonasus*) and Przewalski's horse (*Equus ferus przewalskii*), have actively been introduced into the CEZ after the accident. In addition, despite a lack of protection from contamination, a multitude of wildlife species have been documented to persist inside the CEZ since the time of the accident, although the effects of chronic radiation exposure and absorption on these species remain unclear (Shkvyria and Vishnevskiy 2012).

Immediately after the accident researchers documented negative effects of radiation contamination on wildlife within the CEZ, with direct mortality and population declines observed for several species (IAEA 2006). However, in the absence of humans, populations of several large mammals rapidly rebounded within the CEZ in the first decade after the accident and are now abundant and widely distributed throughout the CEZ (Deryabina et al. 2015, Webster *et al. In press*). Nonetheless, in the three decades since the accident, the long term effects of chronic radiation exposure on wildlife populations remain unclear. Several studies have demonstrated that wildlife within the CEZ continue to accumulate substantial amounts of radiation contamination within their body tissues (Chesser et al. 2001; Ryabokon & Goncharova

2006; Geras'kin et al. 2008; Yablokov 2009) and apex predators can be particularly susceptible to exposure given their trophic status (Fuglei *et al*. 2007; Verreault *et al.* 2008). Some research has found evidence that extensive radiation exposure in Chernobyl has resulted in individual health effects in both passerine bird and small mammal populations (Møller *et al*. 2005; Bonisoli-Alquati *et al.* 2010). However, other researchers have suggested that any individual-level effects have been insufficient to limit population abundances of mammals (Baker 1996; Smith 2008, Deryabina *et al*. 2015).

Furthermore, the majority of research conducted on wildlife within the CEZ has focused on species occupying lower trophic levels in the ecosystem. To date, few studies have been published that have quantified the effects of radiation on abundance of carnivores, including apex predators such as gray wolves (*Canis lupus*) and other large mammals within the CEZ. These species are at risk for increased bioaccumulation of contaminants because of their life history traits, specifically their diet. Møller and Mousseau (2013) reported decreased relative abundance of several predator species in areas of higher radiation density in the CEZ, although their study was limited in spatial and temporal extent and the observed patterns were largely determined by a few outlying points within the red forest, a highly contaminated area (10 $km^2$) of the CEZ that is not representative of the surrounding landscape due to extensive anthropogenic modifications following the accident (Beresford and Copplestone 2011). More recently, researchers have found that several mammalian carnivores, including gray wolves, are not only present in areas of high radiation density within the CEZ (Webster *et al*. *In Press*), but have increased in abundance since the accident to numbers that now exceed those reported for other nature reserves in the region (Deryabina *et al.* 2015). Although these studies suggest that the abundance of many wildlife species have increased in the zone since the evacuation of humans, previous studies have generally relied on indices of abundance rather than explicit measures of animal density, especially for large mammals. Thus, there remains a critical need to develop robust estimates of population density throughout areas varying in contamination density within the CEZ to better elucidate how apex predators and other large mammals may be affected by chronic radiation exposure and the removal of humans from anthropogenic landscapes.

Using molecular scatology methods and spatially explicit capture-recapture modeling, here we present the most robust estimates to date on the density of gray wolves within the Belarusian portion of the CEZ to investigate the population status and relative distribution of wolves within the area, relative to the distribution of contamination and available habitats. We hypothesized that, due to the high mobility and large home range size of gray wolves, density would not be diminished in areas of high contamination density.

## Methods

### *Study Area*

We conducted our study in the 2,162-km$^2$ Polesie State Radioecological Reserve (PSRER) in southern Belarus. The reserve was created in 1988 by the regional government to encompass the portion of the CEZ located within the Belarusian Soviet Socialist Republic (which at that time was a component of the Soviet Union but is now independent Belarus). Approximately 51% of the PSRER is forested, with the remaining 49% composed of abandoned agricultural and developed land (deserted villages, farms, and transportation systems), open fields, and seasonal wetlands. A road system remains throughout the PSRER including a few that are paved or semi-paved, although the majority of roads are graveled or compact dirt. However, few of these roads are maintained or used by humans regularly. Because of the lack of human activities within PSRER, no anthropogenic structures, including roads, were considered barriers to movement. In fact, some species such as wolves have been documented to extensively use the degrading road system as a means of travel (Shkvyria and Vishnevskiy 2012; Deryabina *et al*. 2015)

The PSRER is bisected by the Pripyat River, and human access to the reserve is strictly regulated. More than 8 pecies of mid- to large-sized mammals currently persist within the PSRER, including gray wolves, raccoon dogs (*Nyctereutus procyonoides*), Eurasian bison (*Bison bonasus*), Eurasian boar (*Sus scrofa*), and many others (Shkvyria and Vishnevskiy 2012, Chapter 3). Levels of cesium-137 ($^{137}$Cs) within PSRER remain very high, and as of 2009 soil contaminant densities ranged from 40 kiloBecquerels per square meter (kBq/m$^2$) to >7,500 kBq/m$^2$ across the reserve (Figure 1; Izrael and Bogdevich 2009). Because radionuclides are unevenly distributed across the landscape, exposure rates differ widely among wildlife species due to differences in habitat use, diet, and movement behaviors.

*Sample Collection*

We collected scat samples along 4 discreet road transects, each 30 - 50km in length within the PSRER during autumn 2014 (Figure 3.1). Transects were distributed to encompass much of the PSRER and strategically placed to include a range of $^{137}$Cs contamination densities, varying habitat types, and were separated by a minimum distance of 7.5 km. We surveyed each transect for scat from a vehicle traveling at an average of 30km/hr at least every 5 days during October-November 2014, for a total of 4-6 sampling occasions per transect. Additionally, roads not included in the aforementioned transects were sampled opportunistically throughout the sampling period in an attempt to capture any individuals not sampled along transects and determine a minimum number of individuals within the PSRER. Scats were collected whole in re-sealable plastic bags and frozen upon return to PSRER lab facilities each day in a -20º C freezer. GPS coordinates, estimated age of the scat, and predicted species based on scat morphology were also collected for each sample. At the conclusion of the sampling period, scats were transported to the University of Georgia Savannah River Ecology Laboratory at the Savannah River Site, Aiken, South Carolina, USA under United States Fish and Wildlife Service importation permit number 946712-B for DNA extraction and genotyping.

*Genetic Analysis*

To obtain pure DNA from each scat sample, we collected a subsample from the exterior of the scat by scraping the outermost layer of scat with a razor blade or tweezers and used QIAamp Fast DNA Mini Stool Kits (Qiagen, Hilden, Germany). Extractions were conducted by hand or using the Qiagen Qiacube (Qiagen, Hilden, Germany), a robotic workstation that automates the DNA extraction process. To ensure purified DNA was canid DNA rather than a prey species also present within the scat, all samples were screened for the presence of a short mtDNA sequence (126bp) at the ATP6 locus, which is known to be a reliable marker for species within Carnivora (Chaves *et al*. 2011). Only samples at which the ATP6 mtDNA sequence successfully amplified were utilized in further analysis.

To identify samples to individual, we amplified each sample at 6 microsatellite loci using specific primers previously developed for use in gray wolves: AHT130, C20.253, Cxx.250, C466, FH2096, and PEZ08 (Holmes *et al*. 1995; Ostrander *et al*. 1993; Fransisco *et al.* 1996; and

Creel *et al.* 2003, respectively). Our amplification process utilized 2 PCR cycles that have been optimized for the specific primers used (Holmes *et al.* 1995; Ostrander *et al*. 1993; Fransisco *et al.* 1996; and Creel *et al.* 2003, respectively).

For loci AHT130, C466, C20.253, Cxx.250, and PEZ08, we used a PCR touchdown cycle consisting of a 5-minute initial denaturation step at 95°C followed by 20 cycles of 95°C for 30 seconds, 60°C (decreased 0.5°C per cycle) for 30 seconds, and 72°C for 30 seconds; and 20 cycles of 95 °C for 30 s, 50°C for 30 seconds, and 72°C for 30 seconds, and a final extension step of 72°C for 5 minutes. PCR amplifications were performed in a 12.5 µl reaction [1.25 µl of AmpliTaq Gold 10X PCR Buffer, 1.25 µl of 10xmM bovine serum albumin (BSA), 1.0 µl of 10mM (0.2mM of each) dNTP, 1.5 µl AmpliTaq Gold Magnesium Chloride, 4.0 µl of sample DNA, 0.45 µl each of 10xmM Forward and Reverse Primers and 0.06 µl of AmpliTaq Gold DNA Polymerase].

For loci FH2096 we utilized a PCR cycle of a 10-minute initial denaturation step at 95°C, then a 10-minute secondary denaturation step at 85°C, followed by 33 cycles of 95°C for 1 minute, 64°C for 30 seconds, and 72°C for 45 second, and a final extension step of 72°C for 3 minutes. PCR amplifications for FH2096 were performed in a 17 µl reaction [1.7 µl AmpliTaq Gold 10X PCR Buffer, 1.7 µl 10xmM BSA, 1.5 µl (0.5 units) AmpliTaq Gold Magnesium Chloride, 4.0 µl of sample DNA, 0.45 µl each of 10xmM Forward and Reverse Primers, and 0.06 µl AmpliTaq Gold DNA Polymerase].

We amplified samples 1-5 times and genotyped using fragment analysis methods on an ABI-3130xl Sequencer (company of HW) utilizing a Naurox size standard, described in

DeWoody et al. (2004) but modified to have GTTT on the 5' ends of unlabeled primers. Alleles at each locus were analyzed using GeneMapper version 4.1 software (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and combined to create multi-locus genotypes for each sample. Every genotype was scored independently by two people. Genotypes were then used to assign scats to individuals and estimate probability of identity (the probability of 2 independent samples having the same genotype) using program GenAlEx 6.5 (Peakall and Smouse 2006, 2012) and create spatially explicit capture histories for inclusion in abundance estimators.

*Statistical Analysis*

Based on average home-range sizes reported for gray wolves in the region (600 $km^2$ to 900 $km^2$; Theuerkauf *et al*. 2003) we did not consider transects independent of one another for analysis. Rather, we considered the dataset as a whole in order to estimate density across the PSRER using a spatially explicit capture-recapture framework. This approach (Efford 2004; Efford and Fewster 2013) uses individual spatially explicit capture-recapture (SECR) data sets collected across an array of "detectors" (i.e., live-capture traps, non-invasive scat transects, polygon area searches, etc.) to estimate population density (D, number of individuals within a defined area), the effective survey area (area surrounding detectors which resident animals were likely to be detected), and detection probability (g0, probability of detecting an individual within a defined area) across a user-defined area of integration (hereafter referred to as the state space). All modeling analyses were conducted using the 'secr' package in program R (Efford 2016; R Development Core Team 2008), which uses a maximum-likelihood approach to execute SECR methods. SECR methods typically assume a uniform (i.e. independent) distribution of individuals across the state space. Although gregarious species such as wolves may violate this assumption, models which relaxed the uniformity assumption were not supported. We assumed a closed population during the 6-week sampling period and included spatially explicit capture histories from transects only to estimate density. Individuals captured outside of transects were utilized to quantify the minimum number of wolves within the PSRER.

To determine the appropriate state space needed to increase the accuracy of our SECR modeling, the capture-recapture data were run initially across a null model (detection probability was constrained to be constant across time and individual) several times, each time increasing the stipulated radius of the effective survey area around each transect by 1,000m. While SECR models and resulting estimates of density have been shown to be robust to incorporating too large an effective survey area, they also are sensitive to utilizing too small a survey area (Efford and Fewster 2013). Thus, we expected changes in state space radius would result in significant changes in density estimates (>2% change in estimate), if the resulting survey area was not sufficiently large. Once a large enough state space was incorporated, increasing the radius value further would not result in significant changes in the density estimate (<2% change in estimate). As such, we ran the data across null models with effective survey area radii ranging from 3,000m to 12,000m around each transect, and once the resulting estimates of density were found to deviate <2% over at least 4 radii values, we considered the true effective survey radius the smallest radius value while still ensuring minimal variation in the resulting density estimate. Additionally, we assessed the effects of increasing state space radius on density estimates using full maximum likelihood estimation in package 'secr'. The convalescence of these 2 approaches indicated an appropriate effective survey area around each transect which, because of their non-independence, overlapped in some areas. As such, we cannot make conclusions about densities or detection probabilities along a single transect but rather use the data to model density across the PSRER as a whole.

To do so, we developed a suite of 5 candidate models that incorporated various trends in detection probability across individual (i.e., heterogeneity of individual behaviors), detector (i.e., spatial heterogeneity of transect characteristics), or sampling occasions (heterogeneity over time). The data were fit across the suite of candidate models and then ranked with Akaike Information Criterion (AIC). The top-ranked model based on AIC ranking was then used to predict variation in detection probability across the effective survey area by creating an estimated density surface within program 'secr'. If multiple models were within 2 units of the lowest AIC value, then those models were averaged and the resulting model average was used to make predictions of detection probability. The density surface data layer was then used to identify areas of high density (i.e., detection probability $\geq$0.85) and low density (detection probability of <0.15) throughout the effective survey area and overlaid with derived habitat layers in ArcGIS 10.2.1(ESRI, Redlands, CA) to determine environmental characteristics associated with areas of high and low detection probabilities.

To quantify habitat characteristics across the PSRER, we used GPS technology to demarcate 42 sites located in various habitat types to obtain representative data for each common habitat type that occurs within the reserve. GPS-based positions were used in a supervised classification (ArcGIS 10.2.1, ESRI, Redlands, CA) of LANDSAT imagery obtained from GLOVIS (US Geological Survey Global Visualization Viewer, http://glovis.usgs.gov). We reclassified the content of the resulting raster map into 5 habitat types: pine forest, hardwood [deciduous] forest, seasonal marsh, dry field, and water. We used the Geospatial Modeling Environment (Spatial Ecology LLC, www.spatialecology.com/gme) platform to quantify area-weighted mean soil activity densities of $^{137}Cs$ both within the high-density area and along each survey transect (kBq/m$^2$; based on geo-rectified imagery data from Izrael and Bogdevich [2009]), area of pine and hardwood forest, area of open field, and area of seasonal wetland within the high- and low-density area. Additionally, using Geospatial Modeling Environment, we quantified the number of raster cells of each habitat type within each area of interest (i.e., frequency of habitat occurrence for high- and low-density areas) as well as across the PSRER (i.e., available habitat occurrence). These count data were compared using chi-square ($\chi^2$) tests in program R using the 'stats' package (R Development Core Team 2008). Because radiation data were continuous in nature, average median radiation level was quantified for each raster cell in the high- and low-density areas, as well as across the PSRER. We utilized Shapiro-Wilks tests of normality to determine if these radiation data could be considered normally distributed. Radiation values were then compared between the 2 density areas using a Mann-Whitney U Test in program R using the 'MASS' package (R Development Core Team 2008). This approach allowed us to determine if significant differences existed in the habitat composition and contamination densities of the high- and low-density areas, as well as to compare habitat composition and contamination density of these areas to available habitat within the PSRER.

## Results

We collected 281 wolf scats during the autumn of 2014. Of these, 138 samples (49.1%) were extracted, amplified, and genotyped successfully and 106 unique individuals were identified across the PSRER. Our estimated probability of identity across all 6 loci was $9.0 \times 10^{-7}$, indicating that 6 loci was sufficient to identify each sample to individual. The weighted average $^{137}Cs$ density along each transect was 699.07 kBq/m$^2$ along Transect 1, 815.12 kBq/m$^2$ along

Transect 2, 3018.16 kBq/m$^2$ along Transect 3, and 496.48 kBq/m$^2$ along Transect 4. Further, 27 individuals were detected on Transect 1, 23 were detected on Transect 2, 38 were detected on the Transect 3, and 11 individuals were captured on transect 4 (Figure 3.1). The remaining 7 individuals were identified from opportunistically collected scats not on any of the 4 transects. Some individuals were captured on multiple transects (Figure 3.2), with an average distance between captures of 8.72 km and a maximum distance between captures reaching 21.6 km.

State space radii $\geq$9,000m in the null model resulted in density estimates fluctuating <2%, indicating that a radius of approximately 9,000m encompassed our effective survey area around each transect. Additionally, the maximum likelihood estimation of the influence of increasing the state space radius on estimates of density indicated that the maximum radius at which density estimates were significantly affected was 8,992 m. The convalescence of these 2 methods for determining an adequately large state space provide confidence that our use of a 9,000-m radius for effective survey area is accurate. Thus, we used a state space with radius of 9,000m in all fitted models to estimate density.

The top-ranked model with AIC incorporated heterogeneity in detection probability over time (g0~t; Table 3.1). This model estimated the density of wolves to be 106 individuals/1,000 km$^2$ (95% Confidence Interval [CI] = 78 - 164 individuals/1000 km$^2$; g0 = 0.33; sigma = 0.073) and estimated the realized abundance of individuals across the state space as 265 individuals (95% CI = 197 - 381 individuals, SE = 46). The estimated density surface predicted using the g0~t model resulted in a "high-density area" of 172.64 km$^2$ where detection probability was $\geq$ 0.85 (Figure 3.3). Upon overlaying the high-density area with our derived habitat and radiation data layers, we found that 67.84% of the high-density area fell in areas with $^{137}$Cs contamination density > 2,000 kBq/m$^2$ and the weighted average $^{137}$Cs contamination density across the high-density area was 2,760.41 kBq/m$^2$. Areas associated with detection probabilities $\leq$ 0.15 within the PSRER encompassed 276.97 km$^2$, with a weighted average contamination density of 1,020.50 kBq/m$^2$. For contamination density data of the high and low wolf density areas, Shapiro-Wilks tests revealed a non-normal distribution (high-density area: W = 0.727, p = 0.004576; low-density area: W = 0.803, p = 0.03) and thus the data were subsequently analyzed using non-parametric tests. The Mann-Whitney test revealed a significant difference in contamination density between high- and low-density areas (p = 0.04). This finding indicates areas of high wolf density had significantly higher radiation density than areas of low wolf density, an unexpected trend that we explore further in the discussion below.

Quantification of habitat composition revealed the high wolf density area was comprised of 21.31% pine forest, 12.46% hardwood forest, 23.88% dry field, and 42.35% seasonal marsh. The remaining 7.84% consisted of open water. The low wolf density area was comprised of 39.70% pine forest, 16.15% hardwood forest, 28.46% dry field, 14.18% seasonal marsh, and < 2% open water. The $\chi^2$ tests found a significant difference between the habitat composition of the wolf density areas and available habitat in the PSRER (high-density area $\chi^2$ = 17, df=9, p = 0.046; low-density area: $\chi^2$ = 20, df = 9, p = 0.032). When we directly compared the compositions of the high- and low-density areas to each other, they were only found to be marginally different ($\chi^2$ = 15, df = 9, p = 0.07; Figure 3.4).

## Discussion

Our results, for the first time, utilize molecular scatology to quantify spatial variation in the density of gray wolves in the PSRER using a SECR approach. Further, by associating this spatial variation in wolf density with habitat characteristics, including weighted average $^{137}Cs$ contamination density, we were able to associate trends in wolf density with habitat cover and radiation level. Our estimate of wolf population density, 106 individuals/1000 $km^2$ is high relative to previously reported densities for gray wolves in Europe (8.3 - 83.3 individuals/1,000 $km^2$; Sillero *et al*. 2004). However, previous work has suggested that several wildlife species, especially wolves, likely persist at very high densities within the PSRER compared with other natural park areas within the region (Deryabina *et al*. 2015). As such, our relatively high estimated wolf density is not totally unexpected, and is most likely due to a combination of the lack of human persecution within the PSRER as well as abundant prey species such as red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), wild boar (*Sus scrofa*), and Eurasian beaver (*Castor fiber;* Sidorovich 2009). Indeed, human persecution of gray wolves occurs in natural park areas across Belarus outside of the PSRER,  and includes high quotas for hunting and trapping as well as a state-funded bounty system that pays hunters a small sum for each wolf removed from the landscape (Dr. Dmity Shamovich, personal communication). Thus, it is possible the hunting pressures outside the PSRER suppress wolf abundances and densities in other regions of Belarus, while the unique, inadvertent protection offered by the exclusion of humans from the CEZ allows robust wolf populations to thrive at high densities within the PSRER.

We detected gray wolves throughout the PSRER encompassing areas varying widely in $^{137}Cs$ contamination density, ranging from 75 - 7,499 $kBq/m^2$ (Figure 3.2), suggesting that density of gray wolves is not limited by contamination density within the PSRER. Indeed, we detected the greatest number of individuals on the transect with the highest weighted average contamination density, and this area had the highest estimated wolf density within the reserve.

Statistical analyses revealed a significant difference in contamination density between areas of high and low wolf density, suggesting wolves are utilizing high radiation areas significantly more than low radiation areas. Additionally, this transect is in the central-most area surveyed within the PSRER, farthest from human activity and other anthropogenic pressures (e.g., agriculture) in the surrounding landscape. While elevated wolf density in areas of high radiation is unexpected, it is likely a reflection of the lack of human activity and persecution of wolves in comparison with peripheral areas of the PSRER. Additionally, because of the large space use requirements of wolves it is possible that individuals utilizing territory along the periphery of the PSRER, both inside and outside the protected CEZ, are subjected to increased hunting pressures when outside the PSRER and thus have lower survival, ultimately resulting in the lower densities in that region of the CEZ.

It is important to note, however, that while our sampling design did not include areas of the highest level of contamination density (>7,500 $kBq/m^2$), this area makes up only a small portion of the PSRER (Figure 3.1) and previous research has detected wolves in this area of high contamination density and observed no difference in relative abundance of wolves or other mammals compared to less contaminated areas of the CEZ (Shkvyria and Vishnevskiy 2012; Deryabina *et al*. 2015; Webster *et al*. *In press*). Moreover, our most-contaminated transect encompassed areas of extremely high radiation contamination density (up to 7,499 $kBq/m^2$). As such, we do not believe exclusion of the small area exceeding 7,500 $kBq/m^2$ biased our wolf density estimates reported herein.

Additionally, we detected wolves across several habitat types throughout the PSRER and there was a notable difference in the composition of high-density areas compared to low-density areas. Seasonal marsh and dry field (i.e., unforested habitats) made up 66.23% of the habitat in high-density areas, while the same habitat only comprised 42.64% of low-density areas. Conversely, high-density areas were comprised of only 33.77% pine or hardwood forested habitat, while forested habitats made up 55.85% of low-density areas. Statistical analyses showed that these distributions were significantly different from the proportions of available habitat within the PSRER. Both high- and low-density areas include higher proportions of seasonal marsh and dry field than is proportionally available within the PSRER, indicating that wolves are utilizing these habitats with significantly high frequency. Our findings suggest that wolf densities are highest in marshy, open habitats in the central-most region of the PSRER farthest from human activity. This is not unexpected, and corroborates a trend for human avoidance documented in other European wolf populations (Jedrzejewski *et al*. 2004; Kaartinen *et al*. 2005). Additionally, wolf densities have also been found to vary with access to prey species (Mladenoff *et al*. 1999; Jedrzejewski *et al*. 2004) and as such we believe the suggested high utilization of marshy, open habitats is most likely due to the high abundances of wild boar and beaver in marsh habitats documented to be an important part of wolf diets in PSRER (Sidorovich 2004; Shkvyria and Vishnevskiy 2012). However, our study did not quantify habitat use or preference for gray wolves at the individual or population level within the PSRER. Thus, further study into utilization of various habitat types as well as areas of varying $^{137}$Cs contamination density is needed.

Although our findings suggest wolves are not limited by contamination density, it is important to note that this study did not attempt to quantify individual health effects of $^{137}$Cs contamination exposure. Wolves, as apex predators, are subject to bioaccumulation of contamination in their body tissues (Fuglei *et al*. 2007; Verreault *et al.* 2008), and as such we would expect that if individual health effects due to contamination exposure and absorption were severe enough to diminish individual fitness or survival, then population densities in areas of high contamination density would similarly be diminished; this was a pattern not supported by our data. Furthermore, if contamination exposure and absorption were resulting in severe negative effects throughout the population, then it is unlikely that overall population dynamics would be unaffected. Although it appears that any potential negative individual health impacts operate at a sub-population level and do not limit gray wolves from persisting within areas of significant radiation contamination density, further research is needed to elucidate the health impact of chronic radiation exposure and absorption on gray wolves at both an individual and population scale.

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**Table 3.1:** Akaike Information Criterion (AIC) rankings of fitted spatially explicit capture-recapture models for estimating the density and detection probability of gray wolves within the Polesie State Radioecological Reserve (PSRER).

| Model | Detection Function | K | AIC | AICc | dAIC | AICwt |
|---|---|---|---|---|---|---|
| D~1 g0~t sigma~1 | Half-normal | 8 | 2447.558 | 2449.176 | 0 | 1 |
| D~1 g0~b sigma~1 | Half-normal | 4 | 2467.509 | 2467.939 | 19.951 | 0 |
| D~1 g0~1 sigma~1 | Half-normal | 3 | 2526.196 | 2526.451 | 78.638 | 0 |
| D~1 g0~t sigma~t | Half-normal | 8 | 2698.709 | 2698.884 | 251.151 | 0 |
| D~1 g0~t sigma~b | Half-normal | 4 | 2698.709 | 2698.884 | 251.151 | 0 |

**Notes**: K = number of parameters included in model; dAIC = delta AIC, the difference between the top ranked model and the subsequent models; AICw = assigned weight of model within rankings; D = density; g0 = detection probability as a function of individual heterogeneity; sigma = detection parameter as function of detection method.

**Figure 3.1**: The Polesie State Radioecological Reserve (PSRER), encompasses the portion of the Chernobyl Exclusion Zone within Belarus. Radiation contamination density is spatially heterogeneous throughout the reserve. Four distinct transects highlighted in blue were surveyed multiple times over a 6-week period in Autumn 2014 for gray wolf scats. Scats also were collected from areas outside transects opportunistically. Map derived from imagery reported by Izrael and Bogdevich (2009) and imported to ArcGIS 10.2, georectified, and digitized.

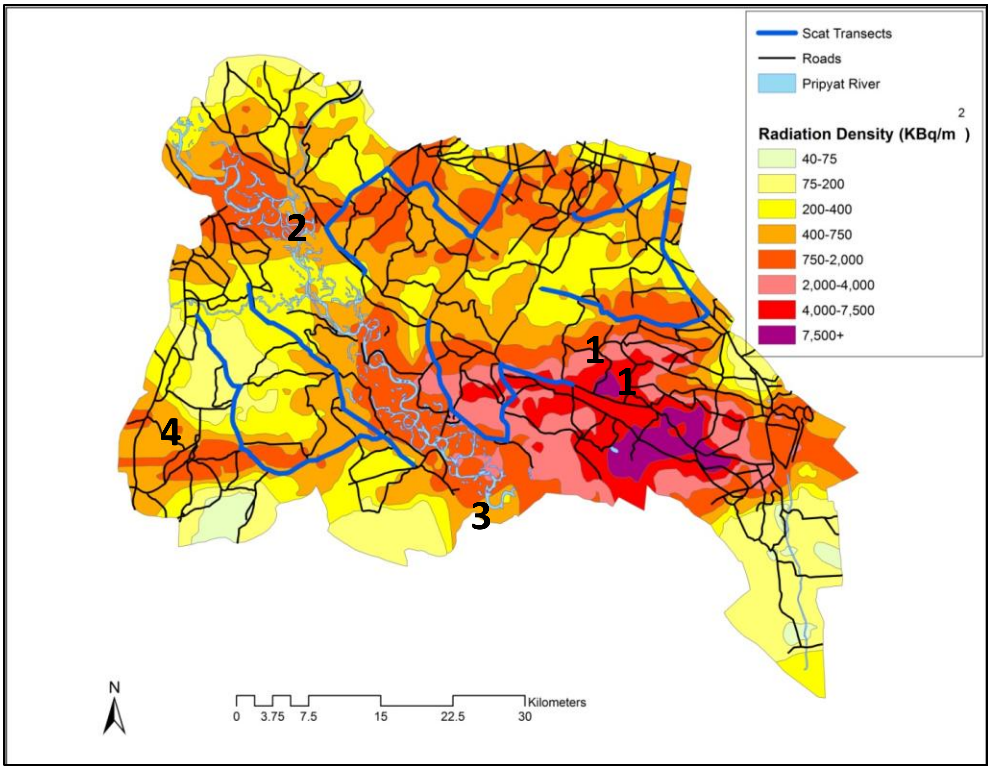


**Figure 3.2:** Scat samples collected across the Polesie State Radioecological Reserve (PSRER). In some cases, individual wolves were detected on multiple transects and in areas of varying $^{137}$Cs contamination density, as exemplified by wolves 1 and 2. Map derived from imagery reported by Izrael and Bogdevich (2009) and imported to ArcGIS 10.2, georectified, and digitized.

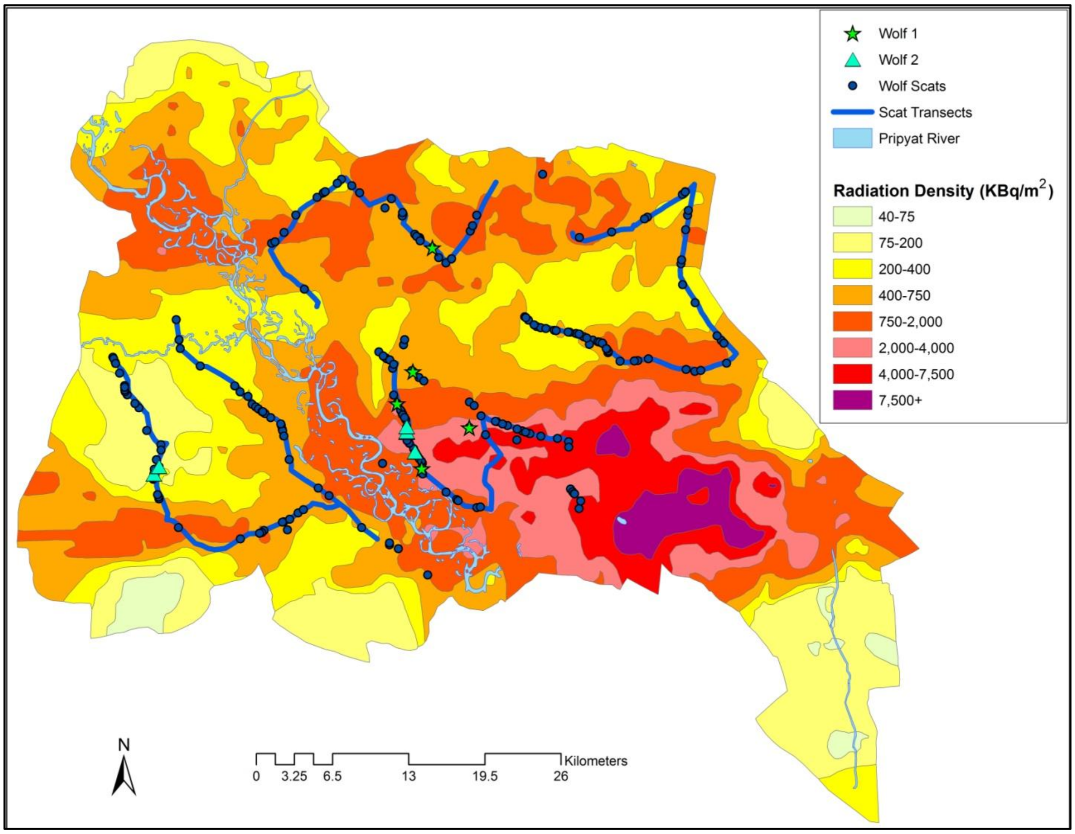


**Figure 3.3**: Estimated Density Surface of gray wolves across effective survey area within PSRER. Areas where detection probability was $\geq 0.85$ were considered a "high-density area" and were used to quantify habitat characteristics and $^{137}Cs$ contamination density associated with high densities of gray wolves.

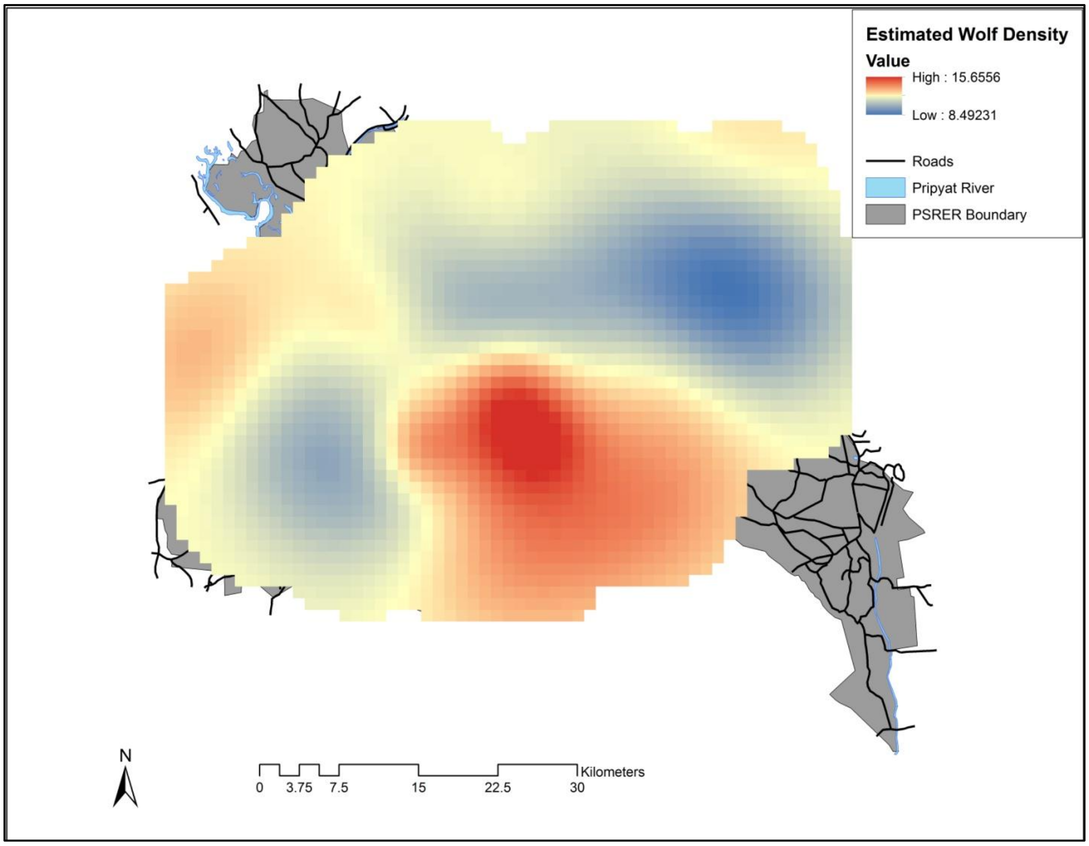
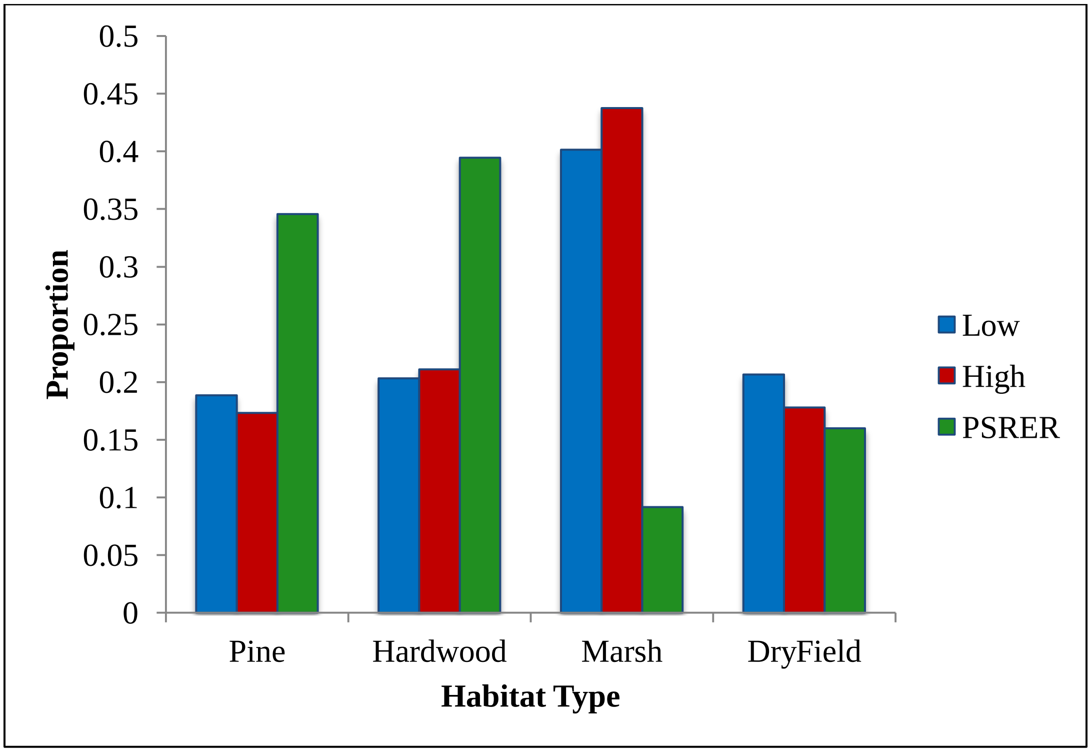


Figure 3.4: Proportions of four habitat types (pine forest, hardwood forest, seasonal marsh, and dry field) in areas of high density, areas of low density, and across the PSRER.



# CHAPTER 4

# CONCLUSIONS

My research provides one of the most quantitatively robust analyses on the distribution of mammalian species as well as density of an apex predator within the CEZ to date. These data suggest that the current distribution of several mid- to large-sized mammals and the density of gray wolves within the CEZ is not limited by $^{137}Cs$ contaminant density. However, I did not examine the health effects of radiation exposure at the individual level, and contaminant densities of $^{137}Cs$ may not directly correlate to absorbed dose rate due to a multitude of factors (e.g., movement, behavior, diet). Long-term, chronic exposure to radiation may possibly affect animal health, although my findings indicate that current levels of exposure are not limiting the distributions of several species nor the densities of gray wolves. Moreover, if individual-level effects were severe, then I would expect a negative correlation between occupancy probability and radiation contaminant density, particularly for species with restricted home-range sizes (e.g., raccoon dog, red fox), and between wolf density estimates and radiation contaminant density. This pattern was not supported by my data.

In Chapter 2, the data revealed that all four species included in occupancy analyses were detected at stations in highly contaminated areas and <500 m from areas with the highest contaminant densities of $^{137}Cs$ in the PSRER ($\geq$7,500 kBq/m$^2$). These species included raccoon dogs and red foxes, which have home-range sizes of only 1.5–2.0 km$^2$ (Drygala and Zoller 2012) and are, therefore, highly influenced by local radiation levels. The occupancy probability for raccoon dogs was positively correlated with radiation contaminant density at both spatial scales measured, as well as with distance to the Pripyat River. A positive correlation between occupancy probability and radiation level, while unexpected, is most likely due to environmental factors not measured in the study (e.g., prey base, interspecific competition). Similarly, red foxes were unaffected by $^{137}Cs$ contaminant density, and had a higher probability of occupying areas farther from the Pripyat River and areas with less seasonal marsh, consistent with habitat requirements for this species (Goldyn *et al*. 2003).

Eurasian boar also have relatively small home ranges (3–15 $km^2$; Baskin and Danell 2003) and thus are likely to be affected by local radiation levels. However, my data do not indicate that populations of Eurasian boar were suppressed in highly contaminated regions of the CEZ, as only distance to the CEZ border was found to influence boar occupancy probability. This correlation, which was negative at both the 250-m and 1,000-m habitat scales, most likely exists because the landscape immediately outside the CEZ is predominantly composed of agricultural croplands. Eurasian boar utilize agricultural crops as a food resource, so areas adjacent to the CEZ border probably support higher densities of boar because they offer increased foraging opportunities (Schley and Roper 2003). Although this trend coincides with boar being more likely to be found in areas of lower radiation, $^{137}Cs$ contaminant density was not found to influence occupancy, and boar were detected at stations in the most-contaminated regions of our study area.

Gray wolves were unique among the species considered in the occupancy analysis in that no measured environmental parameters were found to be influential at either the 250-m or 1,000-m habitat scales. I expected that the effects of $^{137}Cs$ contaminant density on their occupancy probability would be limited because of their large home-range size and high mobility through spatially heterogeneous regions of radiation contamination. Although the data support this hypothesis, I acknowledge that my interpretations for habitat characteristics which determine the occupancy and distribution of gray wolves within the CEZ are limited.

To expand on my estimates of occupancy and distribution derived in Chapter 2, in Chapter 3 I used molecular scatology and spatially explicit capture-recapture models to, for the first time, robustly quantify density of gray wolves throughout the PSRER. I estimated the population density to be relatively high (106 individuals/1000 $km^2$) compared to wolf population densities reported in other parts of Europe (83 individuals/1000 $km^2$; Sillero *et al*. 2004). Estimated wolf densities were highest in the central-most part of the CEZ, near the borders between Belarus and Ukraine, which also corresponds to the area of highest $^{137}Cs$ contamination. This finding, while surprising, is most likely due to habitat or anthropogenic attributes not measured in the scope of this study. Specifically, wolves are subject to high levels of human persecution in the form of hunting and trapping in both Ukraine and Belarus, including a nationwide bounty system on unprotected lands in Belarus for gray wolves (Dr. Dmitry Shamovich, personal communication). As such, because gray wolves have large space-use requirements, individuals using the periphery of the PSRER may be required to move between protected and unprotected lands and thus may be subject to increased hunting pressures that may limit survival, reproduction, and ultimately density of individuals in those areas. Given the distribution and density of wolves observed in the CEZ, my data do not suggest that $^{137}Cs$ densities nor habitat factors are limiting wolf densities within the PSRER. In fact, the high densities I observed in PSRER compared to other regions of Belarus and Europe (Sillero *et al.* 2004, Deryabina *et al*. 2015) suggest wolf populations may be benefiting from the unique protection afforded by the exclusion of humans from the extensive contiguous landscape created by the Chernobyl accident, as well as increased prey abundance found within the CEZ (Deryabina *et al*. 2015).

Overall, my findings indicate that the severity of radiation contamination has no discernible impact on the current distribution of several mammalian species within the CEZ. Rather, other habitat-related and anthropogenic factors (e.g., agricultural lands, human presence) appear to determine occupancy. Similarly, the density of gray wolves is not negatively affected by $^{137}Cs$ contamination densities, nor habitat characteristics within the PSRER. The areas of highest wolf density spanned a range of habitat types including pine forest, deciduous forest, open field, and marsh habitats. While my analysis does not attempt to quantify use of each of these habitats, it does indicate that wolves are found at high densities in a range of habitats found throughout the PSRER. Thus, despite severe impacts on some wildlife immediately after the nuclear accident (Alexakhin and Geras'kin 2013), my results corroborate the conclusions of Baker *et al*. (1996) and Deryabina *et al*. (2015), and suggest that robust populations of numerous mammals now occur throughout much of the CEZ, including areas with radiation levels exceeding 7,500 kBq/m$^2$. The results of the spatially heterogeneous density estimates for gray wolves also indicate that in addition to wolf density not being limited by contaminant density, wolf density is highest in areas of high contaminant density within the CEZ. This trend may be due to hunting pressures outside the CEZ, in which case the unique protection provided by human exclusion in the CEZ seems to hold greater benefit for this population of wolves than potential negative impacts from chronic, long-term exposure to contaminants.

My research contributes to an improved scientific understanding of the long-term ecological consequences of nuclear accidents, and can be applied by policy makers to establish effective management and safety protocols for wildlife in highly contaminated landscapes elsewhere. However, additional research is still needed to assess the potential health impacts of chronic radiation exposure on individuals (e.g., radiation absorption and metabolism over temporal and spatial scales, survival, fecundity) and populations (genetic diversity, dispersal, and functional connectivity to other populations) to more clearly elucidate the long-term effects of exposure to radioactive contaminants.

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