GAME REPORT

Spent Lead Shot Availability, Distribution, and Ingestion by Male Ring-Necked Pheasants and Acute Effects of Lead Pellet Ingestion

Travis Runia
Senior Upland Game Biologist

Alex Solem
Upland Game Resource Biologist

Pittman-Robertson Project W-75-R-56

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Editors:

Tom Kirschenmann
Chief of Terrestrial Resources

Jeff Vonk
Department Secretary

Chad Switzer
Terrestrial Wildlife Administrator

Tony Leif
Division Director

Tanna Zabel
Grants Coordinator

South Dakota Department of Game, Fish and Parks
523 East Capitol Avenue
Pierre, South Dakota 57501

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ABSTRACT

Lead is a non-specific toxin to all vertebrate species, and spent lead shot from hunting activities is one of the most common sources of this environmental pollutant. Birds become exposed to spent lead shot by directly ingesting pellets (primary exposure) or by scavenging carcasses of animals with lead pellets or fragments (secondary exposure). Both acute and chronic effects occur in response to lead exposure including death.

Most research has focused on the availability and ingestion of spent lead shot by waterfowl, especially prior to the 1991 federal ban on use of toxic shot for waterfowl hunting. Less is known about spent lead shot availability and ingestion by upland game species, such as ring-necked pheasants (*Phasianus colchicus*). Limited research has found a 3 - 6% prevalence rate of ingested spent lead shot in gizzards of hunter-harvested upland game birds such as mourning doves (*Zenaida macroura*), northern bobwhite (*Colinus virginianus*), chukar (*Alectoris chukar*), and ring-necked pheasants. We conducted research to determine the availability of spent lead shot and prevalence rate of ingested spent lead shot in wild hunter-harvested ring-necked pheasants on 3 study sites with high shooting intensity in central South Dakota. Two of the sites were licensed shooting preserves where liberal season dates and bag limits are allowed and many pen-raised roosters are released; however, pen-raised birds were censored from the analysis.

We collected 1.3 cm deep soil samples using 76.2 cm X 38.1 cm plots to determine the availability of spent lead shot. In study site 1, soil samples were collected from all landuse types prior to and shortly after the completion of the 2011 - 2012 ring-necked pheasant hunting season. The estimated lead pellet density increased between the first and second sampling period and varied by landuse type. In study site 2, pellet density in the soil was most parsimoniously explained by the distance from hunter blocker locations. In study site 3, pellet density was best explained by proximity to a recreational shooting area on the property and distance from hunter blocker locations.

We collected 123, 168, and 493 gizzards respectively from hunter-harvested wild pheasants from study sites 1, 2 and 3. In study site 1, 8 of 123 or 6.5% (95% CI: 3.3 – 12.3%) of roosters had ingested ≥ 1 lead pellet (range: 1 - 9, mean = 2.38). In study site 2, 9 of 167 or 5.4% (95% CI: 2.9 - 9.9%) of roosters had ingested ≥ 1 lead pellet (range: 1 - 4, mean = 1.55). In study site 3, 17 of 493 or 3.4% (95% CI: 2.16 – 5.45%) of roosters had ingested ≥ 1 lead pellet (range: 1 - 13, mean = 3.24). We collected gizzards from an additional 1,301 hunter-harvested wild pheasants during the 2013 hunting season throughout central and eastern South Dakota from non shooting preserve areas. Of the gizzards collected, 10 of 1,301 or 0.77% (95% CI: 0.41 - 1.41%) of roosters had ingested ≥ 1 lead pellet (range: 1 - 11, mean = 2.44).

We tested liver lead concentrations of all 12 birds that had ingested ≥ 1 lead pellet and 33 birds that had no lead pellets in their gizzard from study site 1. Liver lead concentrations were higher ($P < 0.01$) in birds that had ingested ≥ 1

lead pellet (4.117 ppm) when compared to the 33 reference samples (0.223 ppm).

To better understand the potential acute impacts of ingestion of spent lead shot on pheasants, we gavage-fed 1 and 3 lead pellets to pen-raised hen pheasants. No mortalities were recorded during the 21-day trial, and no significant change in body weight was detected among the test groups or the control group. The mean liver lead concentration for pheasants in the control group was 0.00 ppm. Mean liver lead concentration varied by the number of lead pellets retained at the end of the 3-week trial ($P < 0.01$) with higher liver lead concentrations corresponding with a higher number of pellets retained during the duration of the study.

TABLE OF CONTENTS

Page

Abstract ii

Table of Contents v

List of Figures vi

List of Tables ix

Introduction 1

Objectives 3

Study Area 3

Methods 6

Results 11

Discussion 16

Management Implications 24

Literature Cited 50

LIST OF FIGURES

Figure **Page**

1. Map of study site 1 with landuse type, Lyman County, South Dakota, 2011 - 2012. 25

2. Map of study site 2 with landuse type, Lyman County, South Dakota, 2012. 26

3. Map of study site 3 with landuse type, Lyman County, South Dakota, 2013 27

4. Map of soil sample locations for study site 1, Lyman County, South Dakota 2011 - 2012. 29

5. Map of sample locations for study site 2, Lyman County, South Dakota, 2012 30

6. Custom-made soil sampling device used to sample 1.3 cm of surface soil in non-grassland landuse types 31

7. Sampling method used to collect soil samples from grasslands in study site 2 and all samples in study site 3. First, grass was clipped and removed with a hedge trimmer (picture A), then an 18 volt Dewalt® Reciprocating saw fitted with a 15.2 cm Spyder Scraper® was used to loosen surface soil (picture B) and collect said soil by hand or with a brush and dust pan (picture C). 32

8. Mean liver lead concentration for pheasants with 0, 1, and 2 ingested lead pellets in gizzard at time of harvest from study site 1, Lyman County, South Dakota, 2011. Mean liver lead concentration varied by the number of lead pellets ingested (Kruskal-Wallis test, $P < 0.01$). Pairs with different letters were significantly different ($P < 0.05$) according to multiple tests for all-pairs comparison (Gao et al. 2008). One observation of 9 ingested lead pellets in gizzard with a liver lead concentration of 24.61 ppm not shown. One observation of 3 ingested lead pellets in gizzard with a liver lead concentration of 6.95 ppm not shown 34

Figure **Page**

9. Predicted available lead pellets/ha ± 95% CI for study site 1, Lyman County, South Dakota, 2011 - 2012. Predictions are based on the most parsimonious negative binomial model, (Landuse type + sampling period).....37

10. Mean soil sample volume ± Inter-quartile Range by landuse type from study site 1, Lyman County, South Dakota, during sampling in September 2011 and January 2012. Mean soil sample volume varied by landuse type (Kruskal-Wallis test, P < 0.01). Pairs with different letters were significantly different (P < 0.05) according to multiple tests for all-pairs comparison (Gao et al. 2008). The horizontal black line indicates the target soil sample volume of 2,419 ml according to a 30.5 X 61.0 X 1.3 cm sample. 38

11. Predicted available lead pellets/ha ± 95% C.I. for study site 2, Lyman County, South Dakota, 2012. Predictions are based on the most parsimonious negative binomial model, (intercept + distance blocker).....40

12. Predicted available lead pellets/ha for study site 2, Lyman County, South Dakota, 2012. Predictions are based on the most parsimonious negative binomial model, (intercept + distance blocker). 41

13. Mean soil sample volume ± Inter-quartile Range by landuse type from study site 2, Lyman County, South Dakota, 2012. Mean soil sample volume varied by landuse type (Kruskal-Wallis test, P < 0.01). Pairs with different letters were significantly different (P < 0.05) according to multiple tests for all-pairs comparison (Gao et al. 2008). The horizontal black line indicates the target soil sample volume of 2,419 ml according to a 30.5 X 61.0 X 1.3 cm sample. 42

14. Predicted available lead pellets/ha ± 95% C.I. for study site 3, Lyman County, South Dakota, 2013. Predictions are based on the most parsimonious negative binomial model, (intercept + target shooting area + distance blocker). Predictions are for areas outside the target shooting area only. 44

15. Predicted available lead pellets/ha for study site 3, Lyman County, South Dakota, 2013. Predictions are based on the most parsimonious negative binomial model as applied to areas outside of the target shooting area, (intercept + target shooting area + distance blocker). 45

Figure **Page**

16. Mean volume ± Inter-quartile Range of soil sample by landuse type from study site 3 in Lyman County, South Dakota during sampling in July 2012. Mean soil sample volume varied by landuse type (Mann-Whitney U test, P < 0.01). The horizontal black line indicates the target soil sample volume of 2,419 ml according to a 30.5 X 61.0 X 1.3 cm sample. 46

17. Map of the number of gizzards collected from hunter harvested wild ring-necked pheasants throughout South Dakota, 2013.....47

18. Liver lead concentration in response to the number of lead pellets retained four weeks after lead pellets were gavage-fed to captive hen pheasants. The mean liver lead concentration for pheasants in the control group was 0.00. Mean liver lead concentration varied by the number of lead pellets retained at the end of the 4 week trial (Kruskal-Wallis test, P < 0.01). Pairs with different letters were significantly different (P < 0.05) according to multiple tests for all-pairs comparison (Gao et al. 2008).....48

LIST OF TABLES

Table **Page**

1. Number of released pheasants, released pheasants harvested, wild pheasants harvested, and total pheasants harvested for each study site in Lyman County, South Dakota, 2008 - 2013 28

2. Independent variables used in candidate models to predict available spent lead shot in soil samples for study sites 1, 2 and 3, Lyman County, South Dakota, 2011 - 2013. 33

3. Comparison of negative binomial regression models considered for predicting spent lead shot availability within study site 1, Lyman County, South Dakota, 2011 - 2012. Models are ranked by Akaike's Information Criterion adjusted for small sample size (AIC_c); K is the number of parameters, ΔAIC_c is the difference of each model's AIC_c value from that of the highest ranked model, and *ωi*, is the Akaike weight (sum of all weights = 1.00). 35

4. Predicted available lead pellets/ha for study site 1, Lyman County, South Dakota, 2011 - 2012. Predictions are based on the most parsimonious negative binomial model, (Landuse type + sampling period). 36

5. Comparison of negative binomial regression models considered for predicting spent lead shot availability within study site 2, Lyman County, South Dakota. Models are ranked by Akaike's Information Criterion adjusted for small sample size (AIC_c); K is the number of parameters, ΔAIC_c is the difference of each model's AICc value from that of the highest ranked model, and *ωi*, is the Akaike weight (sum of all weights = 1.00). 39

6. Comparison of negative binomial regression models for predicting spent lead shot availability within study site 3, Lyman County, South Dakota, 2013. Models are ranked by Akaike's Information Criterion adjusted for small sample size (AIC_c); K is the number of parameters, ΔAIC_c is the difference of each model's AICc value from that of the highest ranked model, and *ωi*, is the Akaike weight (sum of all weights = 1.00). All models except the intercept only model include the two-level factor variable target shooting area. 43

Table **Page**

7. Lead shot concentrations in upland habitats, including number of fields sampled, dimensions of each sample, volume of each sample, number of samples, number of lead pellets found and estimated density of lead pellets/ha for each study. A study by Holdner et al. (2004) was conducted on an island used heavily for put and take hunting and target shooting. This study was conducted on properties used for commercial pheasant hunting. All other studies were for managed dove fields. 49

INTRODUCTION

Lead is a non-specific toxin to all vertebrate species (Eisler 1988, Murray et al. 2004) and ingestion of spent lead shot is the most common source of lead exposure in birds (Tranel and Kimmel 2009). Ingestion of lead ammunition (shot pellets, bullets and/or fragments and prey contaminated with lead ammunition) has been documented in over 120 avian species (Tranel and Kimmel 2009). Ingestion of lead causes reduced body function such as anemia, weight loss, reductions in brain function, and reduced oxygen carrying capacity which can cause decreased survival. Prior to the 1991 ban on lead ammunition for waterfowl hunting, an estimated 1.6 to 2.4 million waterfowl died annually from lead poisoning (Friend and Franson 1999). Plautz (2009) estimated 0.34 to 1.66 million mourning doves may potentially die annually from ingesting lead pellets. Both waterfowl and mourning doves are very susceptible to acute lead toxicosis which causes reduced survival after ingestion of as few as 1 - 3 pellets (Schulz et al. 2006, Jordan and Bellrose 1950). In general, occurrence of mortalities is relatively higher in waterfowl than upland game (Friend and Franson 1999).

Less is known about prevalence rates of ingested lead shot and the effects of lead poisoning on most non-migratory upland game birds. Ingested lead shot has been found in chukar (Bingham 2011), northern bobwhite (Keel et al. 2002), ruffed grouse (*Bonasa umbellus*) (Rodrigue et al. 2005), scaled quail (*Callipepla squamata*) (Best et al. 1992), wild turkey (*Meleagris gallopavo*) (Kreager et al. 2007), and ring-necked pheasants (hereafter pheasant) (Calvert 1876,, Hunter and Rosen 1965, Butler et al. 2005). About 9% of hunter-

harvested chukars in Utah had ingested lead pellets (Bingham 2011). Kreager et al. (2008) found prevalence rates of ingested lead pellets of 8 and 34% in a small sample of chukar and pheasants in Canada. Ingestion of lead shot and acute fatal poisoning in pheasants was reported as early as 1876 in Great Britain (Calvert 1876). Butler et al. (2005) found 3.0% of hunter-harvested pheasants on a shooting estate in Great Britain contained at least 1 ingested lead pellet. Hunter and Rosen (1965) reported the lead poisoning death of a pheasant in California which had ingested 29 lead pellets. Gasparik et al. (2012) found reduced reproductive parameters for captive pheasants dosed with lead pellets.

The ring-necked pheasant is the most abundant and most actively hunted non-migratory upland game bird in South Dakota (Switzer 2009). From 2000 - 2012, an average of 166,000 hunters harvested approximately 1.68 million rooster pheasants annually and contributed $164 million annually to the state's economy. Non-toxic shot is required for shotgun hunting of upland game on State Game Production Areas, U.S. Army Corps of Engineers land, U.S. Bureau of Reclamation Game Production Areas managed by South Dakota Department of Game, Fish and Parks (SD GFP), and U.S. Fish and Wildlife Service National Wildlife Refuges and Waterfowl Production Areas which encompass a small proportion (1.6%) of South Dakota's land area. Thus, use of lead shot is legal for pheasant hunting over most of South Dakota. Additionally, shooting preserves licensed with the SD GFP likely have elevated amounts of spent lead shot because they have liberal season length and bag limits and release pen-raised birds for harvest. In 2013, there were 201 licensed shooting preserves

encompassing 80,000 ha where 234,590 released pheasants were harvested. It is unknown if wild pheasants have higher spent lead shot ingestion rates in these areas compared to the rest of the state. Given the social and economic importance of pheasants to South Dakota, information is needed to determine the potential effects of use of lead shot for pheasant hunting in South Dakota, both on preserves and throughout the state.

Objectives

1. Determine the density and distribution of spent lead shot available to upland game birds within areas of high shooting intensity in South Dakota.
2. Determine prevalence rates of ingested spent lead shot by wild ring-necked pheasants within areas of high shooting intensity and throughout South Dakota.
3. Determine the liver lead concentration of wild ring-necked pheasants harvested from an area of high shooting intensity in South Dakota.
4. Determine survival, change in body condition, and liver lead concentration of captive-reared hen pheasants after ingestion of lead pellet(s).

STUDY AREA

For objectives 1 - 3, our study area consisted of 3 separate study sites located in the Northwestern Great Plains Eco-region, specifically the Sub-humid Pierre Shale Plains of South Dakota (Bryce et al. 1996). Study site 1 was 58 ha and located 8 km south and 3 km east of Kennebec, South Dakota (Figure 1). The site was composed of 3 ha of dense, ~ 5 m tall cedar trees (*Juniperus*

virginiana) (4% of the total area), 15 ha of Conservation Reserve Program (CRP) grassland (26% of the total area), 4 ha of food plots (6% of the total area), and 36 ha of no-till cropland (64% of the total area). Food plots were tilled annually and consisted of un-harvested milo (*Sorghum bicolor*). Soils were Millboro silty clay with 0 - 6% slope (Natural Resource Conservation Service 2012).

Study site 2 was 248 ha and located 6 km south and 5 km east of Presho, South Dakota (Figure 2). The site was composed of 68 ha of CRP grassland (27% of the total area), 152 ha of cropland (62% of the total area) and 28 ha of food plots (11% of the total area). Food plots were tilled annually and consisted of un-harvested milo. Dominant soils were Millboro silty clay, and Kolls silty clay soil type with 0 - 6% slope (Natural Resource Conservation Service 2012). All CRP grasslands have been periodically managed by haying or mowing. Some of the land classified as CRP grassland contained rows of recently planted eastern red cedar trees that were less than 2 m tall. The cropland was no-till farmed since 1996 with a rotation of corn (*Zea mays*), soy beans (*Glycine max*), wheat (*Triticum* spp.), sunflowers (*Helianthus annus*), and milo.

Study site 3 was 378 ha and located 3 km east of Vivian, South Dakota (Figure 3). The site was composed of 237 ha of grassland (63% of the total area), 94 ha of food plots (25% of the total area), 32 ha of stream and riparian area (8% of the total area), 9 ha developed (3% of the total area), and 6 ha wetland (1% of the total area). All grasslands have been periodically managed by haying or mowing. Food plots consisted of un-harvested milo or corn with

annual tillage. Soils were predominately Promise Clay with 0-3% slope and Millboro silty clay with 0 - 6% slope.

Study sites 2 and 3 were licensed private shooting preserves with the SD GFP. This designation allows upland game hunting outside the regular upland game season dates, relaxed daily limits and shooting hours, and requires the release of at least as many pen-raised upland game birds as are harvested on the property with a minimum of 600. Upland game bird release and harvest numbers must be recorded by the private shooting preserve operator. All sites have a history of high pheasant harvest (Table 1).

Mean annual precipitation for all study sites is 50.3 cm (South Dakota Climate and Weather 2012a). These sites have a mean annual temperature of 8.3° C (South Dakota Climate and Weather 2012b), with temperatures ranging from 32° C (South Dakota Climate and Weather 2012c) to -15° C (South Dakota Climate and Weather 2012d).

The study area for objective 2 also included all of South Dakota for collection of gizzards from hunter-harvested wild roosters. The study area for objective 4 was the poultry building of the South Dakota State Fairgrounds in Huron, South Dakota.

METHODS

Soil Sampling

We estimated spent lead shot availability to upland game birds by sampling the top 1.3 cm of the soil surface within 76.2 X 38.1 cm sample plots. Landuse type within each study site was digitized using ArcMap 10.0 (Environmental Systems Research Institute, Redlands, CA). Pre-determined upland soil sample locations were distributed in a grid formation throughout the cropland, food plots, trees, and CRP grassland land use types within study site 1 (Figure 4). In study site 1, we collected soil samples once in mid-September 2011 (pre-hunt), and once in January 2012 (post-hunt). The location of each soil sample was recorded with a GPS so the second sampling sequence would occur in approximately the same location, but care was taken not to collect the second sample directly on top of the previous sample. Pre-determined soil sample locations were randomly generated for study sites 2 and 3 to increase spatial variation in samples. The random samples were stratified by landuse type. In study site 2, 250 soil sample locations were generated within CRP grassland, cropland, and food plot land use types and collected during mid-July, 2012 (Figure 5). In study site 3, 450 soil samples were generated in the CRP grassland and 250 soil samples were randomly generated in the food plots and collected mid-July 2013.

Attempts to replicate soil sampling methods described by Castrale (1989) and Douglass (2011) were unsuccessful (see discussion). We primarily used a custom-made soil sampling device to sample the desired 1.3 cm layer of soil

surface (Figure 6). Prior to sampling, loose surface debris was removed. Samples were placed in labeled 3.78 L Ziploc®-style plastic freezer bags. Because the fabricated soil sampling device was ineffective within the grassland landuse type, we used a standard sand style shovel to collect samples from CRP grasslands in study site 1. In an attempt to increase consistency and precision of grassland soil samples, we developed a new method for the subsequent field seasons in study sites 2 and 3. We used an 18 volt cordless DeWalt® reciprocating saw fitted with a 15.2 cm Spyder Scraper® blade to loosen and scrape off the top 1.3 cm of the soil surface within each quadrat (Figure 7). Standing vegetation was cut and removed using bush pruning shears, then the sampling area was outlined by holding the blade vertically. The saw was rotated horizontally and used to carefully loosen the thin 1.3 cm of the soil surface. The loosened soil was collected by hand or with the aid of a small brush and dust pan. This method was used for sampling grasslands in study site 2 and all samples in study site 3.

The volume of each soil sample was recorded, then washed with water through a 2.0 mm test sieve which would strain shot size #9 and larger. Pellets were tested for their magnetic nature to determine ferrous composition (lead or nontoxic).

Prevalence of Spent Lead Shot in Gizzard and Liver Lead Concentration

Gizzards and livers from all hunter harvested wild roosters were collected from study site 1 from 15 October 2011 - 31 December 2011. Gizzards and livers were also collected from an additional 212 roosters harvested from other

locations (non-preserve lands) within 15 km of study site 1 owned by the same commercial hunting operation during the same time period. Gizzards were collected from a portion of the hunter harvested roosters from study site 2 from 19 October - 4 December 2012. Gizzards were collected from a portion of the hunter harvested roosters from study site 3 from 15 September - 15 December 2013. Roosters were classified as adult or young of year using spur length except for study site 3 where age data was not collected (Fredrickson and Trautman 1970). Gizzards were radiographed to determine the presence of metal pellets. Gizzards that contained pellets were necropsied to determine if the pellets were ingested or lodged, and if the pellets were lead or non-toxic. Livers from 45 (12 from birds that had ingested lead pellet(s)) roosters from study site 1 were analyzed by The University of Missouri-Columbia Veterinary Medical Diagnostic Laboratory to determine wet weight liver lead concentration. Only livers without obvious shot damage were submitted to prevent detection of contamination from lead shot residue.

Statewide Gizzard Collections

We collected gizzards from hunter harvested rooster pheasants during the entire 2013 - 2014 pheasant season [12 - 14 October (resident-only season on public land) and 19 October - 5 January (regular season)]. Gizzards were only collected from roosters harvested on lands not licensed as a private shooting preserve with the SD GFP. Gizzards were collected by SD GFP staff and commercial bird processing facilities. Date of harvest, age of bird (young of year vs. adult) (Fredrickson and Trautman 1970), and land ownership where the bird

was harvested were recorded if known (e.g. public vs. private land). Gizzards were examined for ingestion of spent lead shot as described in the previous section.

Experimental Lead Shot Ingestion Study

We conducted a 3-week experiment using captive hen pheasants. We randomly assigned 30 birds to each of 2 experimental groups and a control group. We individually numbered birds with leg bands and placed them in individual cages within an enclosed building. Birds were provided unlimited amounts of commercial poultry food, water, and oyster shell grit. After a 2-week acclimation period, we conducted the trial 16 October - 5 November 2013. We gavage-fed 1 or 3 number 5 lead shot pellets to each bird within each experimental group and compared survival to a control group with no ingested lead pellets. We placed the lead pellets directly into the crop by inserting a 6 mm outside diameter flexible tube into the pheasant's crop and dropped the pellets down the tube. We replicated this process on birds in the control group without adding the lead pellet(s).

Birds were weighed weekly to determine if ingested lead pellets influence pheasant body weight over time. Livers and gizzards were removed from each bird at the end of the study as all birds survived the duration of the study. We radiographed the gizzards at the end of the study for the presence of metal pellets to determine the pellet retention rate. Livers from all 60 treatment birds and 5 control birds were analyzed by the University of Missouri-Columbia

Veterinary Medical Diagnostic Laboratory, Columbia to determine liver lead concentration.

Data Analysis

Statistical analyses were conducted in R (version 2.5.12) (R Core Team 2012) using packages MASS (Venables and Ripley 2002), MuMIn (Barton 2013), binGroup (Zhang 2012), pastecs (Ibanez 2013), and nparcomp (Konietschke 2012). We used a generalized linear mixed model with a negative binomial distribution to evaluate the influence of landuse type, sample period, and spatial variables on the density and distribution of spent lead shot in soil (Table 2). A list of *a priori* models was developed by considering reasonable combinations of these variables. We used an information theoretic approach to estimate the support for models (Burnham and Anderson 2002). Models were ranked and evaluated using Akaike's Information Criterion corrected for small sample size (AICc). We ranked our models based on differences between AICc for each model compared to the minimum AICc model, Akaike weights (w_i) to assess the weight of evidence for each model, and the sum AICc weight for each variable (Burnham and Anderson 2002). We created maps of model-predicted lead pellet density using the raster calculator tool in ArcMap 10.0 to apply model predictions to study sites 2 and 3.

The soil volume data was not normally distributed, so we used a Kruskal-Wallis or Mann-Whitney U test to test for differences in soil volume among or between landuse types. If a difference among groups was detected, nonparametric multiple tests for all-pairs comparisons were conducted (Gao et al.

2008). We tested for a difference in liver lead concentration among birds that had ingested different numbers of lead pellets using a Kruskal-Wallis test. If a difference among groups was detected, nonparametric multiple tests for all-pairs comparisons were conducted (Gao et al. 2008). We reported prevalence of ingested lead shot in gizzards with Wilson Score confidence intervals. A Fisher's exact test was used to test for differences in ingested lead shot prevalence between young of year birds and adults and between birds harvested on shooting preserves and non-preserve areas. For the experimental ingestion study, a paired t-test was used to determine if initial pheasant weight was similar to pheasant weight at the end of the 2-week acclimation period.

RESULTS

Study Site 1

Eight of 123 (6.5%: 95% CI: 3.3 - 12.3%) roosters had ≥ 1 ingested lead pellet in the gizzard (range: 1 - 9, mean = 2.38, SE = 0.98). Including roosters not harvested on the designated study site, 12 of 335 (3.6%: 95% CI: 2.1 - 6.2%) roosters had ingested ≥ 1 lead pellet (range: 1 - 9, mean = 2.17, SE = 0.65). Eleven of 236 (4.6%: 95% CI: 2.6 - 8.2%) young of year birds and 1 of 97 (1.0%: 95% CI: 0.2 - 5.6%) adult birds ingested ≥ 1 lead pellet. The proportion of young of year birds that had ingested ≥ 1 lead pellet was not significantly different than adults ($P = 0.12$). Liver lead concentrations were higher ($P < 0.01$) in birds that had ingested ≥ 1 lead pellet (4.12 ppm) when compared to the 33 reference samples (0.22 ppm). Mean liver lead concentration was higher for pheasants

that had 1 or 2 ingested lead pellets at time of harvest when compared to the 33 reference samples (Figure 8).

We collected 315 soil samples prior to the pheasant hunting season and 323 soil samples after the completion of pheasant hunting season, for a total of 638 samples. Forty-eight samples of the 315 soil samples collected prior to the pheasant hunting season contained ≥ 1 lead pellet totaling 75 pellets, and 41 of the 323 soil samples collected after the pheasant hunting season contained at least 1 pellet totaling 161 pellets. Our most parsimonious model explaining pellet density in the soil contained the variables landuse type and sample period, accounted for > 80% of the AICc weight, and was the only model considered competitive (Table 3). Results suggest pellet density was substantially higher in trees than the other 3 landuse types and nearly doubled during the post-hunt period (Figure 9, Table 4).

Soil sample volume from the collected soil samples in study site 1 varied by landuse type ($P < 0.01$). Only the sample volume for trees appeared to be substantially different in magnitude from the other 3 landuse types (Figure 10).

Study Site 2

Nine of 167 (5.4%: 95% CI 2.9 - 9.9%) gizzards collected from hunter harvested wild roosters had ≥ 1 ingested lead pellet(s) in the gizzard (range: 1 - 4, mean = 1.55, SE = 0.34). Four of 103 (3.88%: 95% CI 1.5 - 9.6%) young of year birds and 5 of 63 (7.94%: 95% CI 3.4 – 17.3%) adult birds had ingested ≥ 1 lead pellet. One pheasant of unknown age was collected but its gizzard did not contain ingested lead pellet(s). No significant difference was detected in the

ingested lead pellet prevalence rates between adults and young of year birds (P = 0.30).

We collected 746 soil samples of which 93 had ≥ 1 lead pellet. We found 107 total lead pellets. Our most parsimonious model to estimate spent lead shot density in the soil included the variable distance from blocker (Table 5). The top AICc ranked model contained 3 additional parameters but only differed by 1.04 AICc units which indicates the model had uninformative parameters. Similarly, the model including distance from blocker and landuse type had a similar AICc value as the most parsimonious model, but contained 2 additional parameters. Summed model weights for models containing distance to blocker was 1.0 indicating high importance. Predicted spent lead shot pellet density increased as distance to blocker declined and ranged from about 2,000 pellets/ha to 11,000 pellets/ha (Figure 11, Figure 12). The soil sample volume varied by landuse type for study site 2 ($P < 0.01$) and was significantly higher in CRP versus cropland ($P < 0.01$) or food plot ($P < 0.01$) (Figure 13). Samples from cropland and food plots were not significantly different from each other ($P = 0.67$).

Study Site 3

Seventeen of 493 (3.44%: 95% CI: 2.16 - 5.45%) gizzards collected from hunter harvested wild roosters had ≥ 1 ingested lead pellet(s) in the gizzard (range: 1 - 13, mean = 3.24, SE = 0.97). We collected 685 soil samples of which 149 had ≥ 1 lead pellet. The samples contained 413 total lead pellets. After soil sample collection had begun, we were informed that recreational shotgun target shooting had occurred in close proximity to where we were collecting samples.

To account for this additional source of spent lead shot while modeling, we included a categorical variable for areas within and outside the terminal ballistic range of shot pellets from the target shooting area for all models. Our most parsimonious model predicting spent lead shot density in the soil included distance to blocker and proximity to the recreational shooting area (Table 6). This model contained 4 fewer parameters than the top AICc ranked model but only differed by 5.73 AICc units. The second ranked model contained 1 additional parameter than the most parsimonious model, but had a similar AICc value, again indicating presence of an uninformative parameter. Model-predicted spent lead shot density decreased as distance from blocker location increased (Figure 14 and 15). Predicted spent lead pellet density within the recreational shooting area was 16.86 times higher (95% CI: 7.15 - 51.65) than outside the area. The soil sample volume varied by landuse type ($P < 0.01$) and was significantly higher in samples collected in the food plots compared to the samples collected in the grass (Figure 16).

Statewide Pheasant Gizzard Collection

We collected gizzards from 1,301 wild hunter-harvested pheasants during the 2013 - 2014 pheasant hunting season throughout South Dakota (Figure 17). The landuse type of harvest was unknown for 35 birds and age of bird was unknown for 46 birds. Ten of 1301 (0.77%: 95% CI: 0.42 - 1.41%) gizzards collected from roosters harvested statewide had ≥ 1 ingested lead pellet(s) in the gizzard (range: 1 - 11, mean = 2.4, SE = 0.98). One of 150 (0.66%: 95% CI: 0.12 - 3.68%) gizzards collected from areas where lead shot was not allowed had ≥ 1

ingested lead pellet(s) in the gizzard. Nine of 1,116 (0.81%: 95% CI 0.42 - 1.52%) gizzards collected from area where toxic shot was allowed had ≥ 1 ingested lead pellet(s) in the gizzard (range: 1 - 11, mean = 2.56, SE = 1.08). Seven of 777 (0.90%: 95% CI 0.44 - 1.85%) gizzards collected from young of year pheasants had ≥ 1 ingested lead pellet(s) in the gizzard (range: 1 - 11, mean = 4.67, SE = 3.18). Three of 478 (0.63%: 95% CI 0.21 - 1.83%) gizzards collected from adult pheasants had ≥ 1 ingested lead pellet(s) in the gizzard (range: 1 - 3, mean = 1.43, SE = 0.30). There was no significant difference (P = 0.75) in prevalence rates between adult and young of year pheasants.

All Gizzards Combined From Study Sites 1-3 and Statewide Gizzard Collection

A total of 2,296 gizzards were examined for the presence of ingested lead pellet(s). The prevalence rate of pheasants that ingested ≥ 1 lead pellet was higher for pheasants harvested on licensed shooting preserves (3.94%: 95% CI 2.70 - 5.71%) than for non-preserve areas (1.34%: 95% CI 0.89 - 2.03%) (P = .009).

Experimental Lead Pellet Ingestion Study

After a 2 week acclimation period, the mean weight of the birds was similar to the initial weight. (initial weight = 782g, 2-week weight = 827g, P < 0.001). All pheasants survived the duration of the 21-day study.. Two birds from the control group and 2 birds from the group given 1 pellet were excluded from the analysis due to injuries sustained from escaping their cage. Percent change in body weight from the date pellets were administered to the end of the study

was not significantly different between the control group and birds that retained at least 1 pellet (P = .08). The control group gained 2.81% (95% CI 0.83 - 4.77%) while the birds that retained pellets gained 5.12% (95% CI 3.42 - 6.82%).

Of the 118 gavage-fed lead pellets, 62 (52%: 95% CI: 44 - 61%) were retained through the end of the study. For the group given 1 pellet, 15 of 28 pellets (54%: 95% CI: 36 - 70%) were retained through the end of the study. For the group given 3 pellets each, 47 of 90 pellets (52%: 95% CI: 42 - 62%) were retained through the end of the study.

DISCUSSION

Spent lead shot availability within our study sites varied by landuse type and sampling period for study site 1, and by distance from blocker locations for study sites 2 and 3. Available lead pellets ranged from as few as 1,450 pellets/ha to as high as 625,000 pellets/ha, well within the range reported for upland habitats in the literature (Table 7). Our highest estimates were within an area used for recreational target shooting. Holdner et al. (2004) reported spent lead shot within the top 10 cm of soil on an island used for put and take pheasant hunting and recreational target shooting in Canada. They found spent lead shot abundance ranging from 0 pellets/ha to 205,100,000 pellets/ha based on only 14 samples. The very high spent shot abundance was attributed to intense target shooting, but the results cannot be directly compared to our study because we only looked at the top 1.3 cm of surface soil, a depth considered available to upland game. For fields used for hunting only, they reported 422,222 lead

pellets/ha. The only other studies that we are aware of that looked at available spent lead shot for upland habitats were for managed mourning dove fields.

Immediate post-hunt weather conditions were only favorable for soil collection for study site 1. Consistent with managed dove fields studied by Lewis and Legler (1968), Anderson (1968), Castrale (1989), Schulz et al. (2002), Best et al. (1992), and Douglas (2011); spent lead shot availability increased substantially post-hunt for study site 1. This suggests that current year gunning contributes disproportionately more to the availability of spent shot than the accumulation from past hunting seasons. Lead shot may move slowly downward in the soil profile over time making it unavailable to upland game.

Available spent lead shot varied by landuse type for study site 1 only. We did not specifically investigate the potential effects of tillage on the availability of spent lead shot. However, tillage regimes did vary by landuse type for all study sites. Food plots were the only landuse type tilled annually, but had similar amounts of available lead pellets as the no-till cropland. Lead pellet availability was much higher in the trees and CRP grassland landuse types than for food plots or cropland. The property owner indicated that hunting pressure was most intense around the trees, which were adjacent to the CRP grassland. This may explain the high pellet densities in these landuse types. Available pellets in the annually tilled food plots were low, but comparable to the no-till cropland. For study sites 2 and 3, lead shot abundance was highest near areas repeatedly used by the “blocker” hunters. The owners of study sites 2 and 3 indicated that blockers often get a majority of the shots while pheasant hunting. Overall,

available lead pellet density was related to hunting pressure within each site, not landuse type or tillage regime. Douglas (2011) found available lead shot did not vary by tillage regime within managed dove fields in North Carolina.

Similar to Schulz (2002), we measured the volume of soil samples to determine precision of sampling and potential sources of variation. Schulz (2002) noted an observer bias, but we did not record observer information because of the large number of observers involved (20+ for each site). Soil sample volumes varied by landuse for each study site. Samples collected from trees contained higher volumes than for samples from cropland, food plot, or CRP grassland within study site 1. The soil beneath the trees was mostly free of organic debris. This may have allowed larger soil samples to be collected in this landuse type. Samples from the other landuse types often contained organic debris which was removed before the soil volume was recorded. The mean sample volumes ranged from 2057 ml to 2629 ml, which is not substantially different than the desired 2,419 ml per sample. We were surprised that the samples from the CRP grassland (2,245 ml mean volume) were close to the target value of 2,419 ml, given we used a sand shovel to collect the samples. However, we do not believe the sand shovel was a precise way of skimming the top 1.3 cm of surface soil from a quadrat.

Soil volume was higher for samples collected from CRP grassland than from cropland or food plots for study site 2. This could have resulted from using a different collection method in the CRP grassland than in the cropland or food plot landuse types. The method using the reciprocating saw was used in the

CRP grassland and may have contributed to the larger sample size. The range of mean sample volume ranged from 2,219 ml to 2,768 ml which is quite close to the desired sample size. Samples from the CRP grassland may have contained organic matter that was too small to be efficiently removed prior to measuring the volume. The fluffy nature of the small organic matter may have increased the volume of air in each sample, thus inflating the observed sample size. Samples from food plots and cropland often contained large pieces of organic matter that was easy to remove prior to measuring the volume. However, samples from CRP grassland were significantly lower than for samples from food plots for study site 3, when the same collection method was used for both landuse types. The samples were not substantially different in magnitude (CRP grassland = 2,998 ml, food plot = 2,627 ml) and were slightly higher than the desired sample size of 2,419 ml. While some variation was observed due to landuse type and/or collection method, overall soil sample volume was reasonably close to the desired amount indicating our methods of collecting samples were acceptable. We recommend the method using the reciprocating saw as it was the fastest and easiest method for all landuse types.

We are unaware of any other study that examined the prevalence rate of ingested lead pellets for only wild male ring-necked pheasants. Prevalence rates of ingested lead pellets in the gizzards of hunter-harvested wild roosters from study sites 1, 2 and 3 (3.4 - 6.5%) were slightly higher than estimates reported for male and female pheasants from shooting estates in Great Britain (3.0%, n = 437) (Butler et al. 2005). Unlike our samples, the samples from Butler et al.

(2005) likely contained a mixture of wild and captive-raised birds, and were collected during the spring and during the fall and winter hunting season. Our estimates were lower than reported from Kreager et al. (2008) (34%, n = 47 captive-raised pheasants) from an intensely hunted island in Canada where high amounts of target shooting also contributed to the accumulation of spent lead shot. Our estimates for pellet ingestion were higher than scaled quail (0.4%) and northern bobwhite (1.8%) in New Mexico (Best et al. 1992), but not higher for chukars (9.20%) in Utah (Bingham 2011).

Our estimates of prevalence rates of ingested lead shot from study sites 1, 2, and 3 were higher than for mourning doves collected in Tennessee (1.2%) (Lewis and Legler 1968), Indiana (2.5%) (Castrale 1991), New Mexico (0.2%) (Best et al. 1992), Missouri (0.3%) (Schulz et al. 2002), or Maryland, Virginia, North Carolina and South Carolina combined (2.4%) (Kendall and Scanlon 1979). Locke and Bagley (1967) found 6.5% of mourning doves had ingested lead shot, but only 62 birds were collected.

The prevalence rate of ingested lead shot in hunter-harvested mourning doves may be lower because ingestion of as few as 1 - 2 pellets causes death within 2 days (Schulz 2006), thus removing those birds from the harvestable population. Little is known about the survival of pheasants after ingestion of lead shot, but Gasparik et al. (2012) did not report any mortality after gavage-feeding captive pheasants 2-6 lead pellets per week for 10 weeks. Our study did not document any mortality 3 weeks after 1 or 3 lead pellets were gavage-fed to captive hen pheasants. This suggests that the prevalence rate of ingested spent

lead shot could be closer to the true ingestion rate for pheasants than for mourning doves. However, results of captive bird studies with constant environmental conditions must be interpreted with caution as changes in environmental conditions can increase mortality rates (Buerger et al. 1986).

The prevalence rate of ingested lead pellets was much lower for birds collected throughout South Dakota (0.77%) vs. those collected from the very heavily hunted study sites (3.4 - 6.5%) and higher for birds collected from hunting preserves (3.94%) vs. birds not collected from hunting preserves (1.34%). While we did not estimate the lead shot availability throughout South Dakota, it is assumed lower overall than the study sites. Our results suggest that ingestion of spent lead shot by pheasants may increase with pellet availability. Unexpectedly, this relationship has not been demonstrated for mourning doves in the wild when comparing studies of pellet ingestion and availability (Lewis and Legler 1968, Castrale 1991, Best et al. 1992, Schulz et al. 2002), nor has this relationship been established in captive studies (Schulz et al. 2007, Plautz et al. 2011). We did not have an adequate gizzard sample size to determine whether ingestion of spent lead shot occurred more frequently on areas where lead shot was allowed for hunting versus areas where non-toxic shot was required.

Unlike most other experimental lead shot ingestion studies, we did not observe any mortality during our 3-week treatment period. Waterfowl typically die after ingestion of 1 - 2 lead pellets (Jordan and Bellrose 1950). Buerger et al. (1986) found reduced survival in mourning doves with as few as 1 gavage-fed lead pellet. Similarly, Schulz et al. (2006) found reduced survival of captive

mourning doves with as few as 2 gavage-fed pellets. Our captive-raised hen pheasants (786.7 g) were much larger than the mourning doves studied by Schulz et al. (2006) (112 - 127 g). Schulz et al. (2006) found a 2.4 - 3.9% decrease in hazard of death for every 1 g increase in pre-treatment body weight for mourning doves. The larger size of pheasants may have reduced the effects of acute lead toxicosis. Body mass change during the post-treatment period did not vary by experimental group in our study which is consistent with Gasparik et al. (2012).

Liver lead levels at the end of our 21-day post-treatment period increased with the number of pellets retained. The mean liver lead concentration was 2.35 PPM for pheasants that retained 2 pellets which is similar to what Gasparik et al. (2012) reported (2.98 PPM) for pheasants given 2 pellets per week for 10 weeks. Gasparik et al. (2012) also reported an increase in mean liver lead concentration as the number of pellets administered increased. For pheasants that retained all 3 pellets they were given, mean live lead concentration was 3.90 PPM, a level considered diagnostic of lead toxicosis (Locke and Thomas 1996, Bauck and LaBonde 1997). Schulz et al. 2006 reported that treated doves that survived the 21-day treatment period had liver lead levels of 3.44 PPM while doves that died during the treatment period had a mean level of 49.20 PPM. The highest liver lead level we observed was in a hunter-harvested rooster pheasant from Study Site 1 which had a liver lead PPM of 24.61 and 9 ingested lead pellets in its gizzard. Another rooster from the same site had 3 ingested lead pellets and a liver lead PPM of 6.95.

Results from our captive pheasant study suggest that pheasants are less susceptible to the acute effects of lead poisoning than mourning doves. It is difficult to determine the overall effect of lead poisoning from ingested lead shot on the wild pheasant population. Although the pen trial did not reveal any mortality within 21 days from the ingestion of 1 - 3 pellets, the average number of pellets found in hunter-harvested birds, detrimental effects could still impact wild birds. For instance, multiple studies have shown that the effects of acute lead toxicosis can be much more severe when birds are exposed to cold temperatures (Kendall et al. 1981, Buerger et al. 1986). This suggests that results of pen trials conducted in controlled settings may not fully reveal the expected effects on wild birds. Because exposure to lead impacts the nervous system, lead poisoning could cause behavioral changes in birds, making them more susceptible to predation, even if direct mortality from acute toxicosis does not occur. Finally, ingestion of lead shot may cause reduced reproductive potential in wild pheasants (Gasparik et al. 2012).

MANAGEMENT IMPLICATIONS

Spent lead shot is available and ingested by wild ring-necked pheasants in South Dakota, which could cause reduced survival and reproduction; however, this was not documented in the short monitoring period of captive individuals within our study. Ingestion of spent lead shot occurs more frequently within shooting preserves where lead shot deposition rates are assumed to be higher than non-preserve areas. Pheasants do not appear to be as sensitive to the effects of acute lead toxicosis as mourning doves or waterfowl, but much is unknown on the impacts on free-ranging pheasants. The availability of spent lead shot declines over time, so spent lead shot availability could be reduced by the use of non-toxic shot. Future research should assess the chronic effects of lead shot ingestion on the survival and reproduction of free-ranging wild ring-necked pheasants.

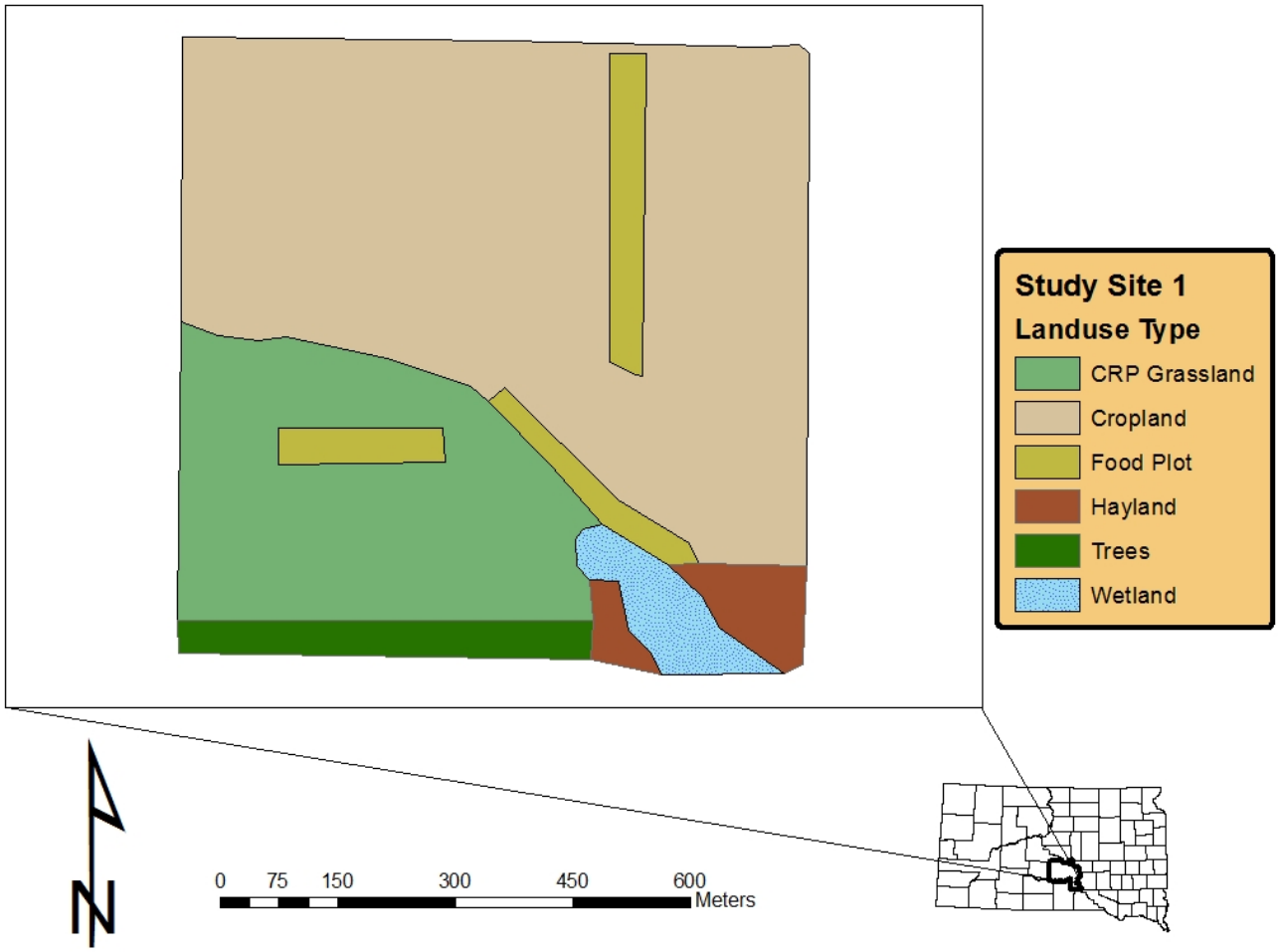

Figure 1. Map of study site 1 with landuse type, Lyman County, South Dakota, 2011 - 2012.

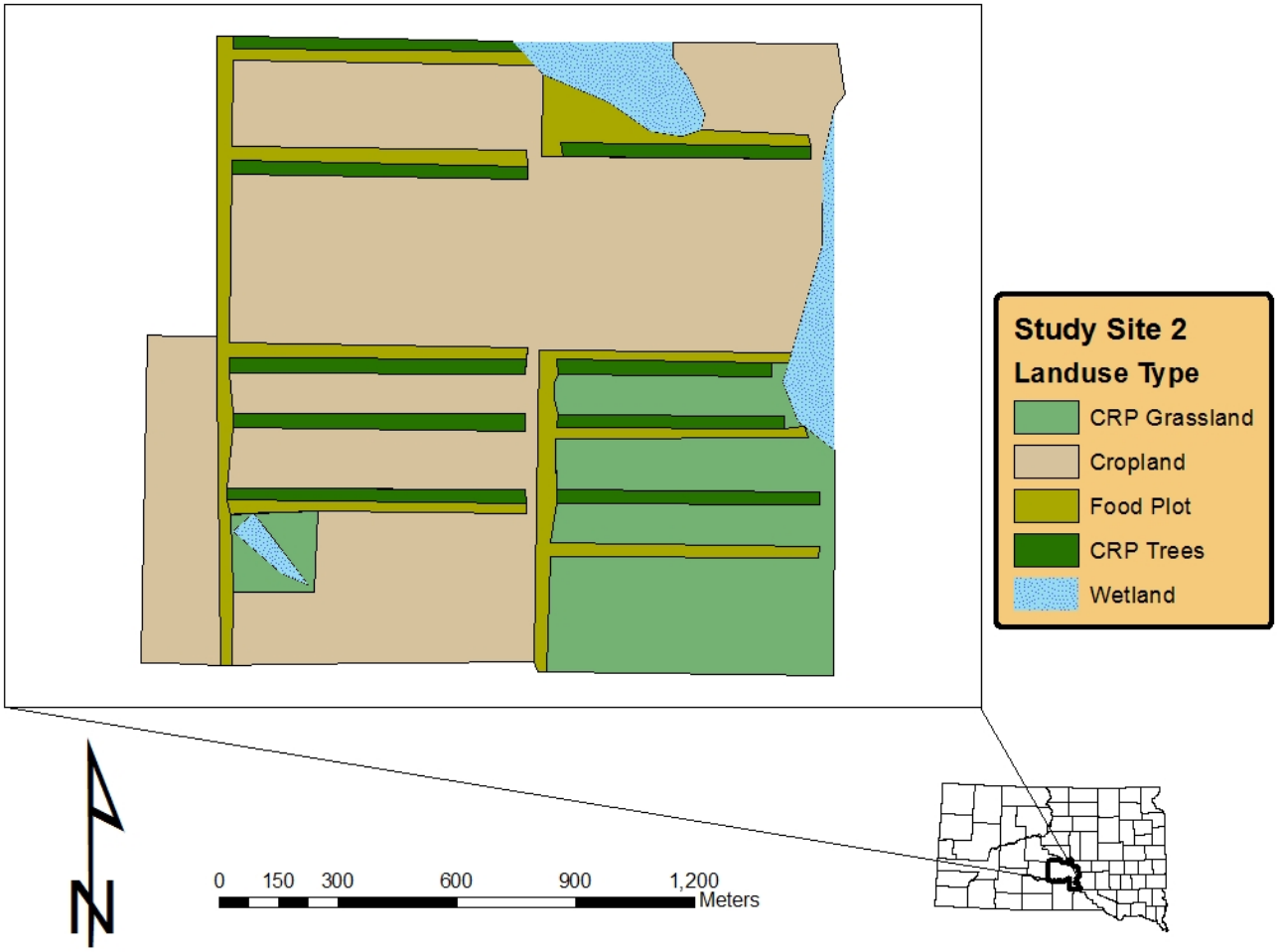

Figure 2. Map of study site 2 with landuse type, Lyman County, South Dakota, 2012.

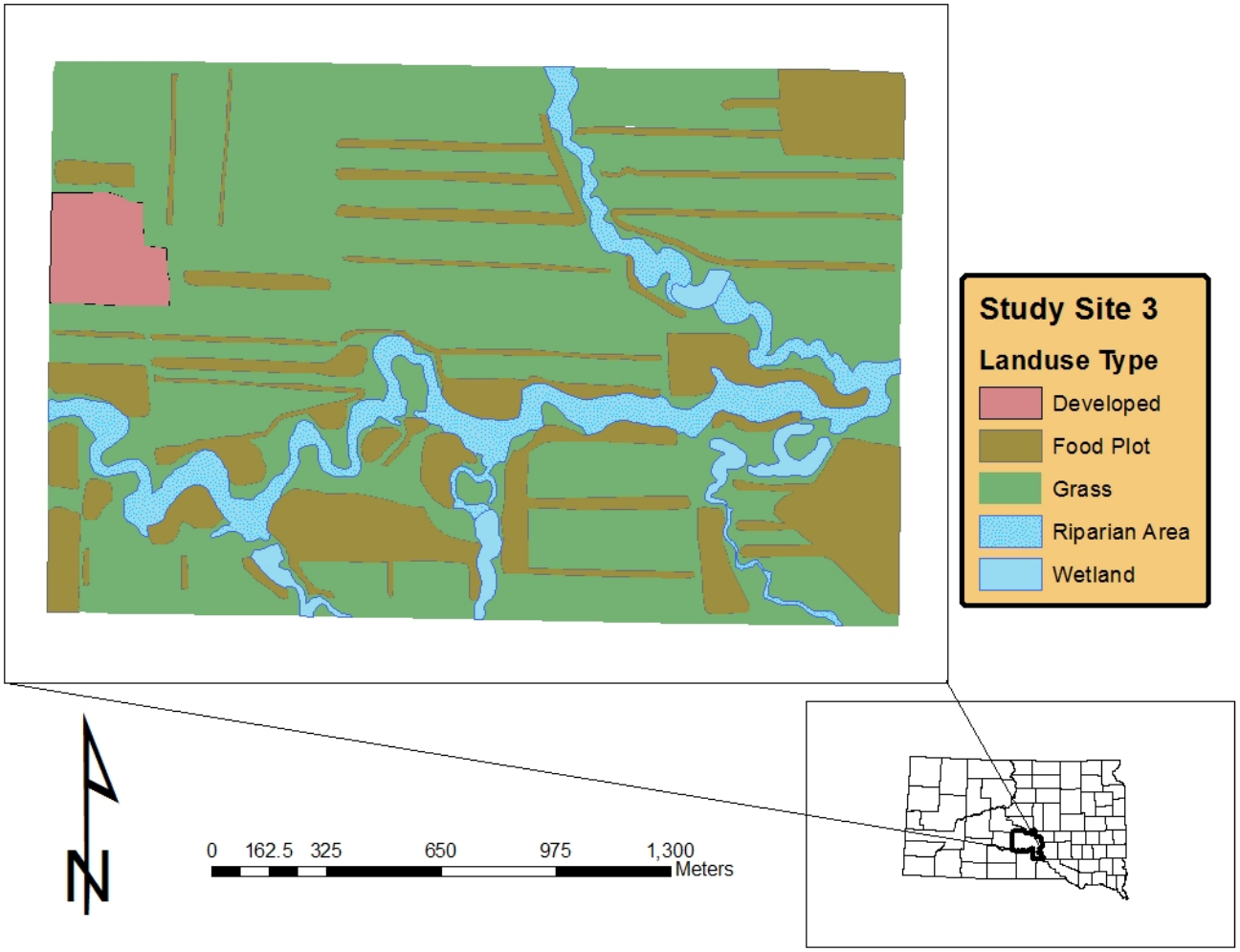

Figure 3. Map of study site 3 with landuse type, Lyman County, South Dakota, 2013.

Table 1. Number of released pheasants, released pheasants harvested, wild pheasants harvested, and total pheasants harvested for each study site in Lyman County, South Dakota, 2008 - 2013.

Year	Site	# Released Pheasants	# Released Pheasants Harvested	Wild Pheasants Harvested	Total Pheasants Harvested
2008 - 2009	Study site 1	-	-	472	472
	Study site 2	1589	405	1043	1448
	Study site 3	1602	1111	486	1597
2009 - 2010	Study site 1	-	-	515	515
	Study site 2	1550	524	977	1501
	Study site 3	1595	743	815	1558
2010 - 2011	Study site 1	-	-	352	352
	Study site 2	1880	566	1159	1725
	Study site 3	2188	889	1221	2110
2011 - 2012	Study site 1	-	-	254	254
	Study site 2	2250	759	1353	2112
	Study site 3	1825	992	817	1809
2012 - 2013	Study site 1	-	-	-	-
	Study site 2	2375	965	524	1489
	Study site 3	3133	1670	465	2135

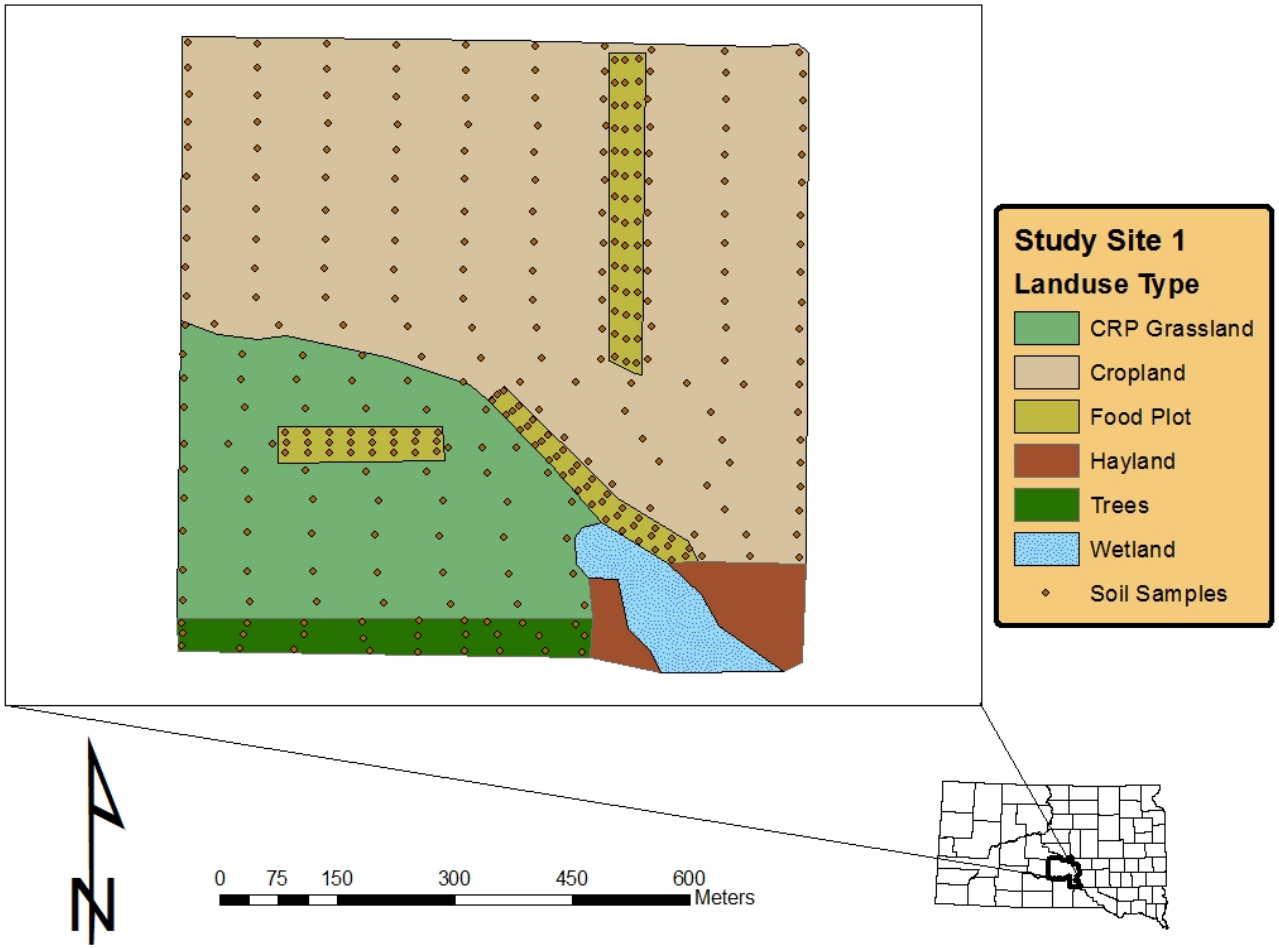

Figure 4. Map of soil sample locations for study site 1, Lyman County, South Dakota 2011 - 2012.

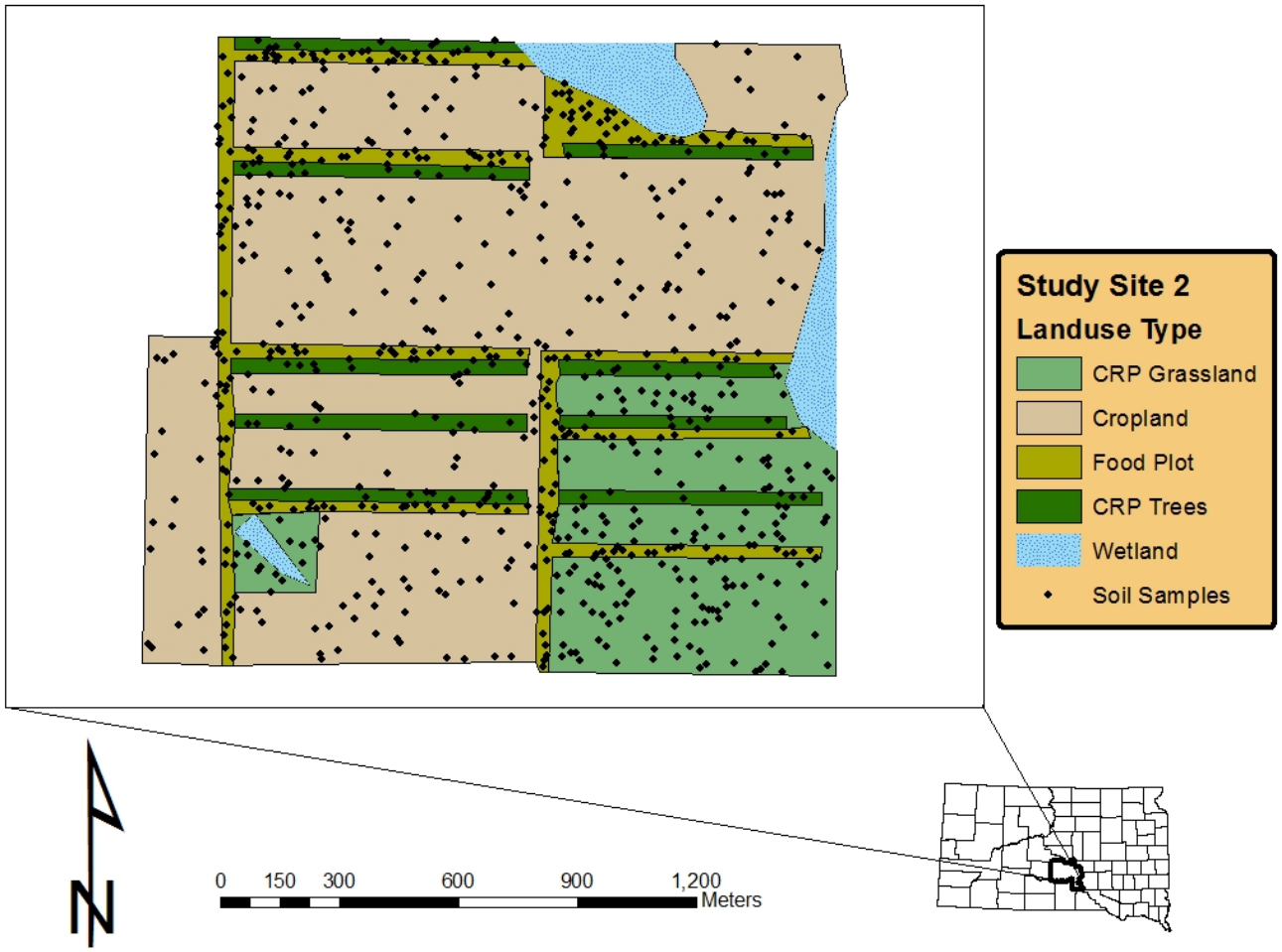

Figure 5. Map of soil sample locations for study site 2, Lyman County, South Dakota, 2012.

Figure 6. Custom-made soil sampling device used to sample 1.0 cm of surface soil in non-grassland landuse types.

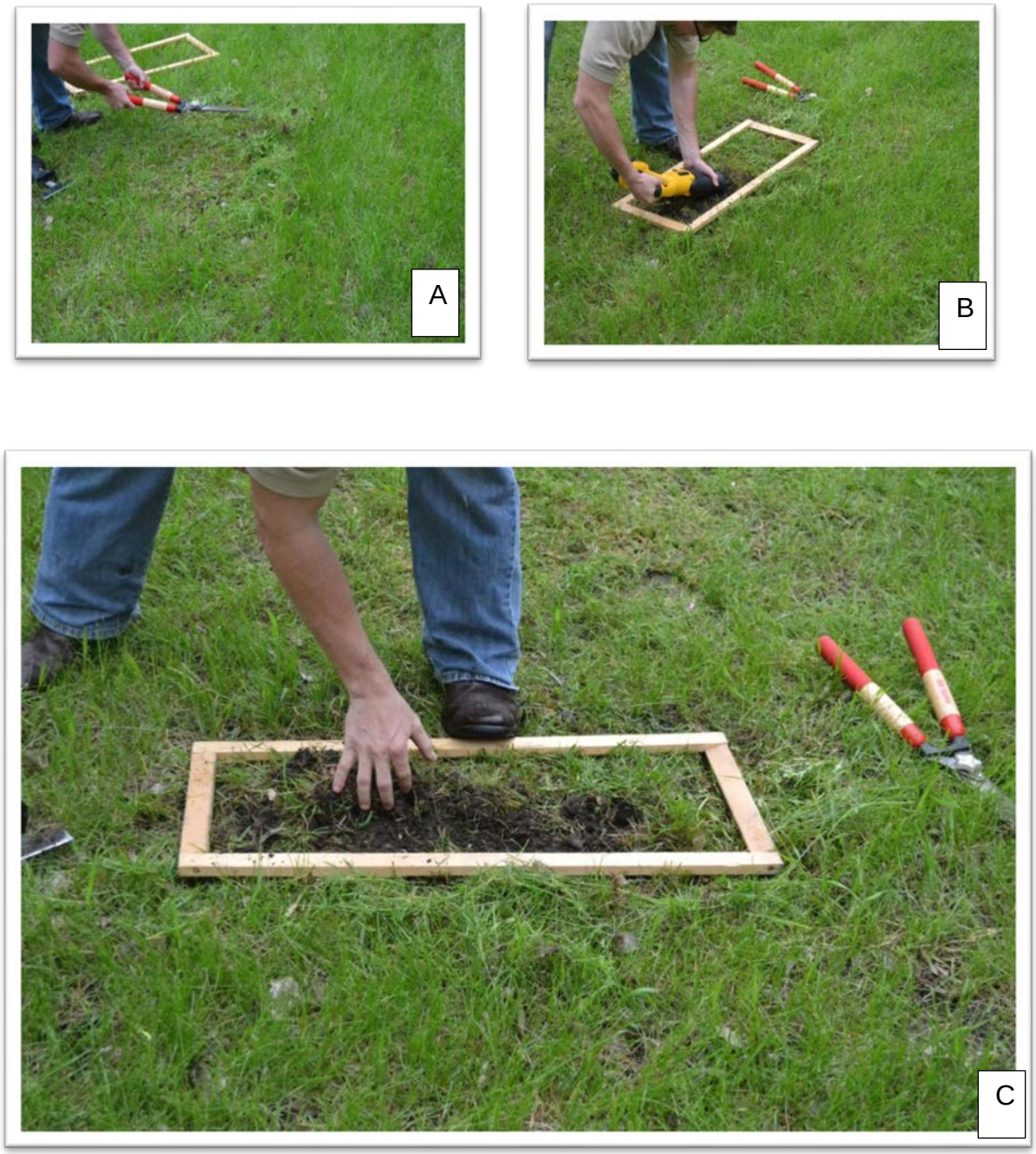

Figure 7. Sampling method used to collect soil samples from grasslands in study site 2 and all samples in study site 3. First, grass was clipped and removed with a hedge trimmer (picture A), then an 18 volt Dewalt® Reciprocating saw fitted with a 15.2 cm Spyder Scraper® was used to loosen surface soil (picture B) and collect said soil with a brush and dust pan (picture C).

Table 2. Independent variables used in candidate models to predict available spent lead shot in soil samples for study sites 1, 2 and 3, Lyman County, South Dakota, 2011 - 2013.

Site	Variable	Definition
Study Site 1	Landuse type	A four level factor of landuse including CRP grassland, cropland, food plot, and trees.
	Sample period	A two level factor indicating when the soil samples were collected. Pre-hunt was in September 2011, Post-hunt was January 2012.
	Trees	A two level factor of landuse including trees, and all other landuses combined.
	Distance to food plot	The distance from the center of the landuse food plot to the soil sample location.
	Distance to trees	The distance from the center of the landuse trees to the soil sample location
Study Sites 2 & 3	Landuse type	A three level factor of landuse including CRP grassland, cropland, and food plot for Study Site 2, a two level factor of CRP grassland and food plot for Study Site 3.
	Distance to blocker	The distance from the line or point where the hunters blocking are usually located to the soil sample location. See study area section for map of blocker locations.
	Distance to walker	The distance from the lines where the hunters walking are usually located to the soil sample location. See study area section for map of walker locations.
	Target shooting area	Two level factor for areas impacted by recreational shotgun target shooting in study site 3. The area was dilineated by buffering the center of the target shooting area by a distance of 229 m, the ballistic range for shot pellets.

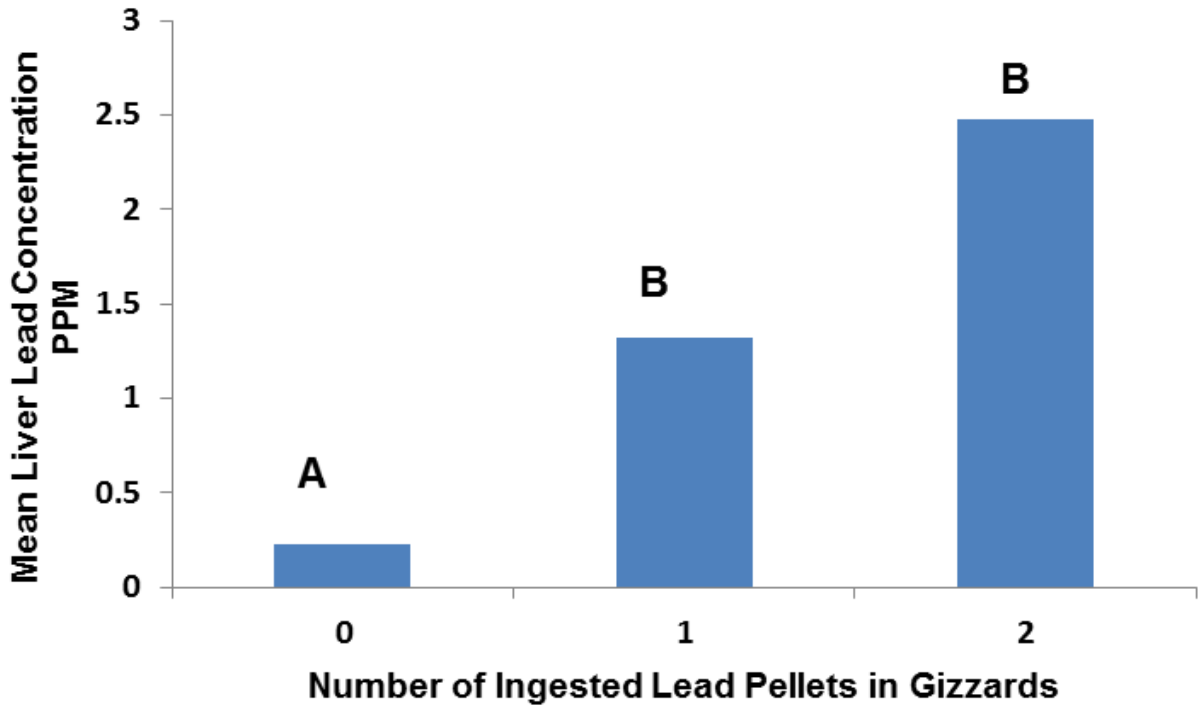

Figure 8. Mean liver lead concentration for pheasants with 0, 1, and 2 ingested lead pellets in gizzard at time of harvest from study site 1, Lyman County, South Dakota, 2011. Mean liver lead concentration varied by the number of lead pellets ingested (Kruskal-Wallis test, $P < 0.01$). Pairs with different letters were significantly different ($P < 0.05$) according to multiple tests for all-pairs comparison (Gao et al. 2008). One observation of 9 ingested lead pellets in gizzard with a liver lead concentration of 24.61 ppm not shown. One observation of 3 ingested lead pellets in gizzard with a liver lead concentration of 6.95 ppm not shown.

Table 3. Comparison of negative binomial regression models considered for predicting spent lead shot availability within study site 1, Lyman County, South Dakota, 2011 - 2012. Models are ranked by Akaike's Information Criterion adjusted for small sample size (AIC_c); K is the number of parameters, ΔAIC_c is the difference of each model's AIC_c value from that of the highest ranked model, and *ωi*, is the Akaike weight (sum of all weights = 1.00).

Model	**K**	**AICc**	**Δ AICc**	**ω*i***
Landuse type + sample period	5	643.20	0.00	0.83
Landuse type	4	647.12	3.92	0.12
Landuse type * sample period	8	648.54	5.34	0.05
Distance to trees + sample period	3	667.43	24.23	0.00
Trees + sample period	3	672.64	29.44	0.00
Distance to trees	2	673.16	29.96	0.00
Trees	2	677.08	33.88	0.00
Distance to food plot + sample period	3	775.44	132.24	0.00
Distance to food plot	2	778.47	135.27	0.00
Sample period	2	798.42	155.22	0.00
Intercept only	1	802.47	159.27	0.00

Table 4. Predicted available lead pellets/ha for study site 1, Lyman County, South Dakota, 2011 - 2012. Predictions are based on the most parsimonious negative binomial model, (Landuse type + sampling period).

Landuse type	# Samples	Sample period	Pellets/ha	SE
CRP Grassland	39	Pre-Hunt	12,286	3,342
Food Plot	104	Pre-Hunt	2,650	780
Cropland	144	Pre-Hunt	1,886	548
Trees	27	Pre-Hunt	91,915	21,668
CRP Grassland	43	Post-Hunt	22,212	5,521
Food Plot	105	Post-Hunt	4,791	1,289
Cropland	148	Post-Hunt	3,410	899
Trees	27	Post-Hunt	166,172	38,221

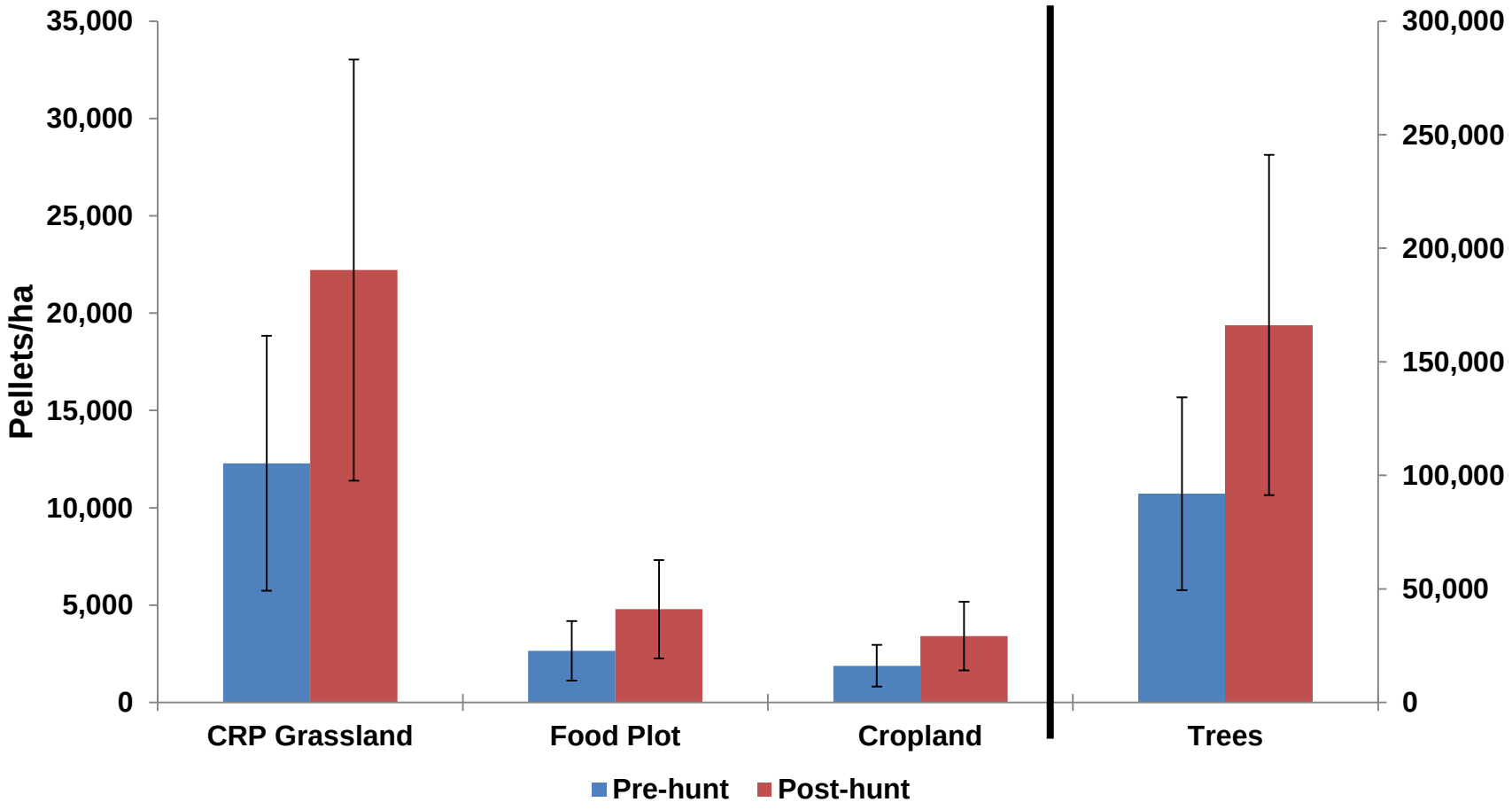

Figure 9. Predicted available lead pellets/ha ± 95% CI for study site 1, Lyman County, South Dakota, 2011 - 2012. Predictions are based on the most parsimonious negative binomial model, (Landuse type + sampling period).

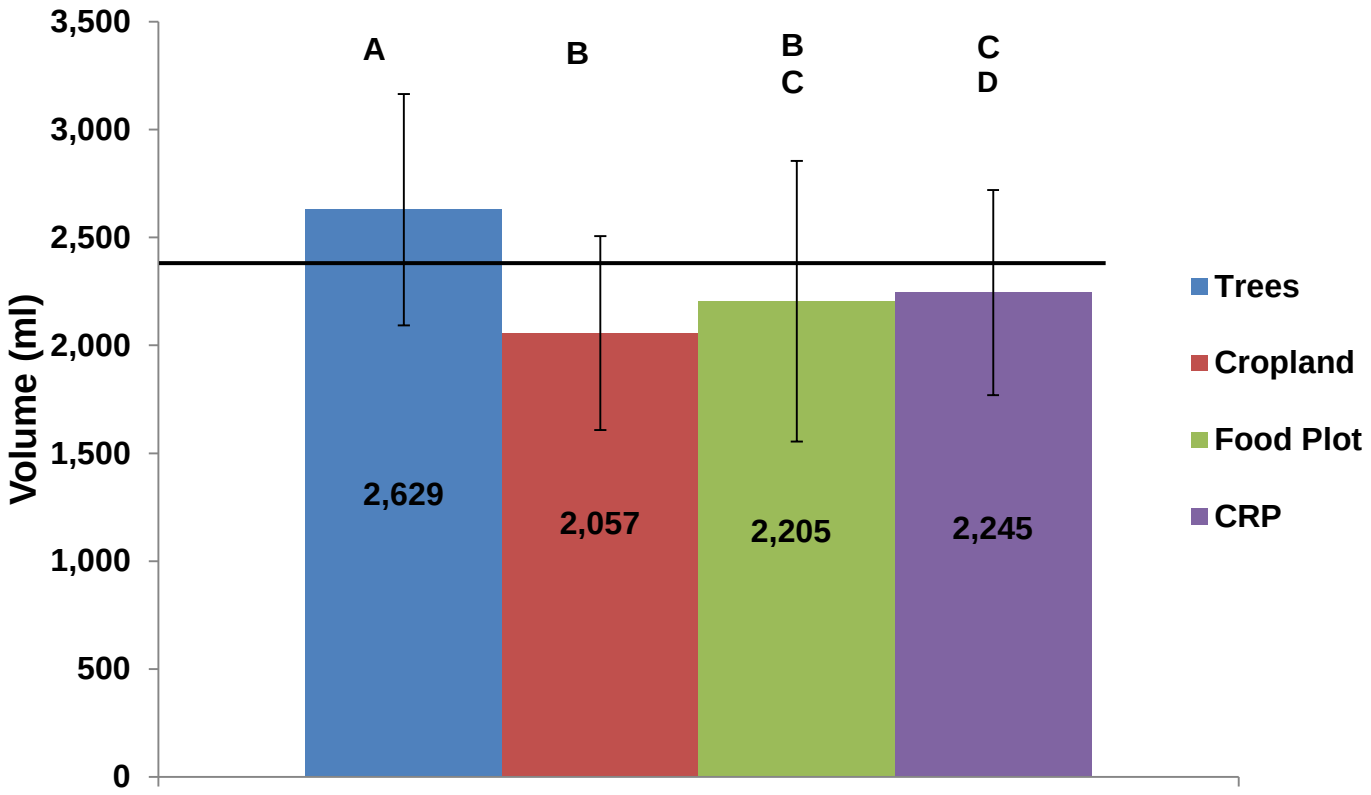

Figure 10. Mean soil sample volume ± Inter-quartile Range by landuse type from study site 1, Lyman County, South Dakota, during sampling in September 2011 and January 2012. Mean soil sample volume varied by landuse type (Kruskal-Wallis test, $P < 0.01$). Pairs with different letters were significantly different ($P < 0.05$) according to multiple tests for all-pairs comparison (Gao et al. 2008). The horizontal black line indicates the target soil sample volume of 2,419 ml according to a 30.5 X 61.0 X 1.3 cm sample.

Table 5. Comparison of negative binomial regression models considered for predicting spent lead shot availability within study site 2, Lyman County, South Dakota. Models are ranked by Akaike's Information Criterion adjusted for small sample size (AIC_c); K is the number of parameters, ΔAIC_c is the difference of each model's AICc value from that of the highest ranked model, and *ωi*, is the Akaike weight (sum of all weights = 1.00).

Model	**K**	**AICc**	**Δ AICc**	***ωi***
Distance blocker + distance walker + landuse type	5	635.55	0.00	0.42
Distance blocker	2	636.59	1.04	0.25
Distance blocker + landuse type	4	636.99	1.44	0.20
Distance walker + distance blocker	3	638.30	2.75	0.11
Distance walker * landuse type + distance blocker * landuse type	9	641.73	6.18	0.02
Intercept only	1	648.21	12.66	0.00
Distance walker	2	649.34	13.79	0.00
Landuse type	3	649.95	14.40	0.00

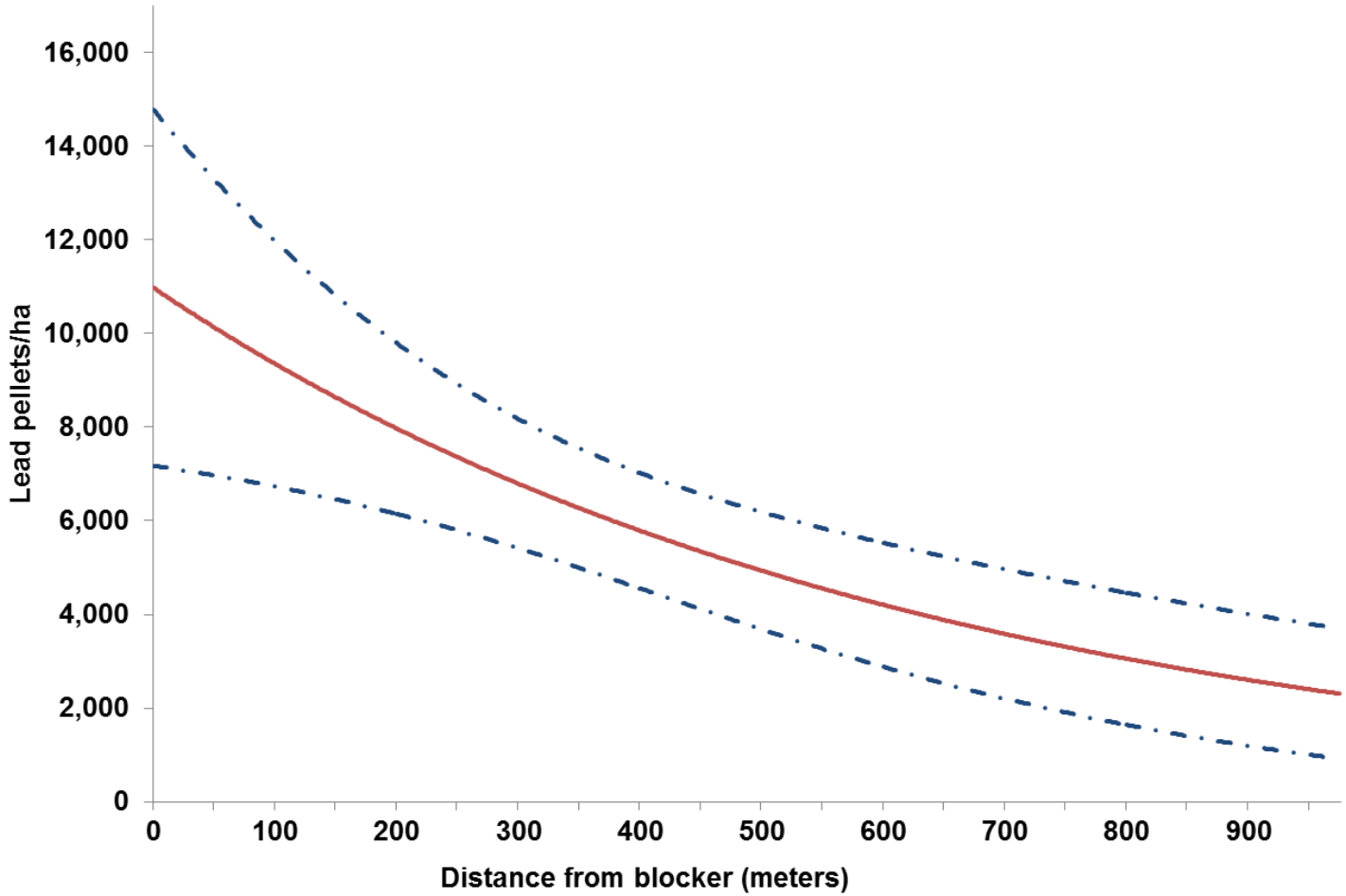

Figure 11. Predicted available lead pellets/ha ± 95% C.I. for study site 2, Lyman County, South Dakota, 2012. Predictions are based on the most parsimonious negative binomial model, (intercept + distance blocker).

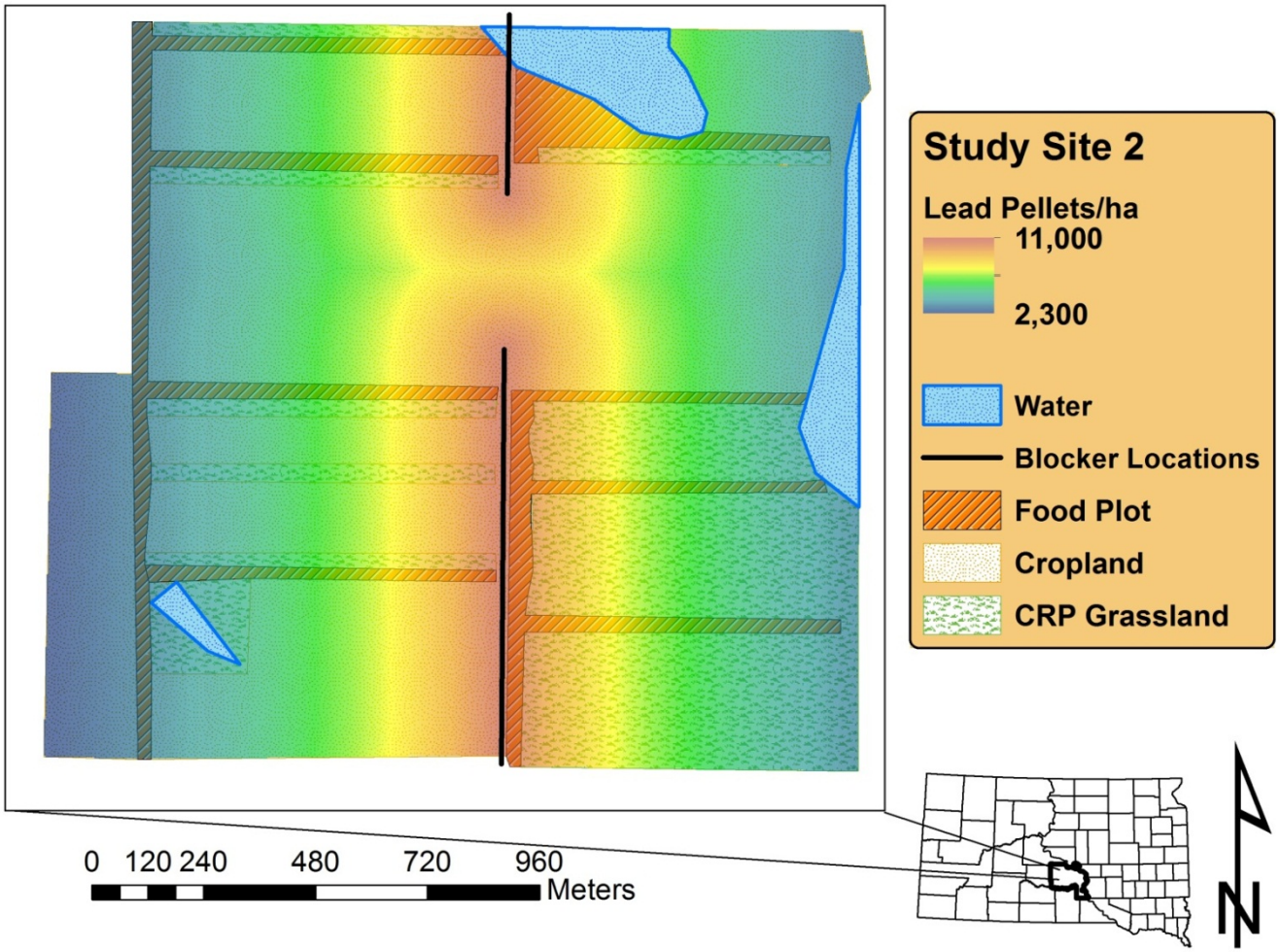

Figure 12. Predicted available lead pellets/ha for study site 2, Lyman County, South Dakota, 2012. Predictions are based on the most parsimonious negative binomial model, (intercept + distance blocker).

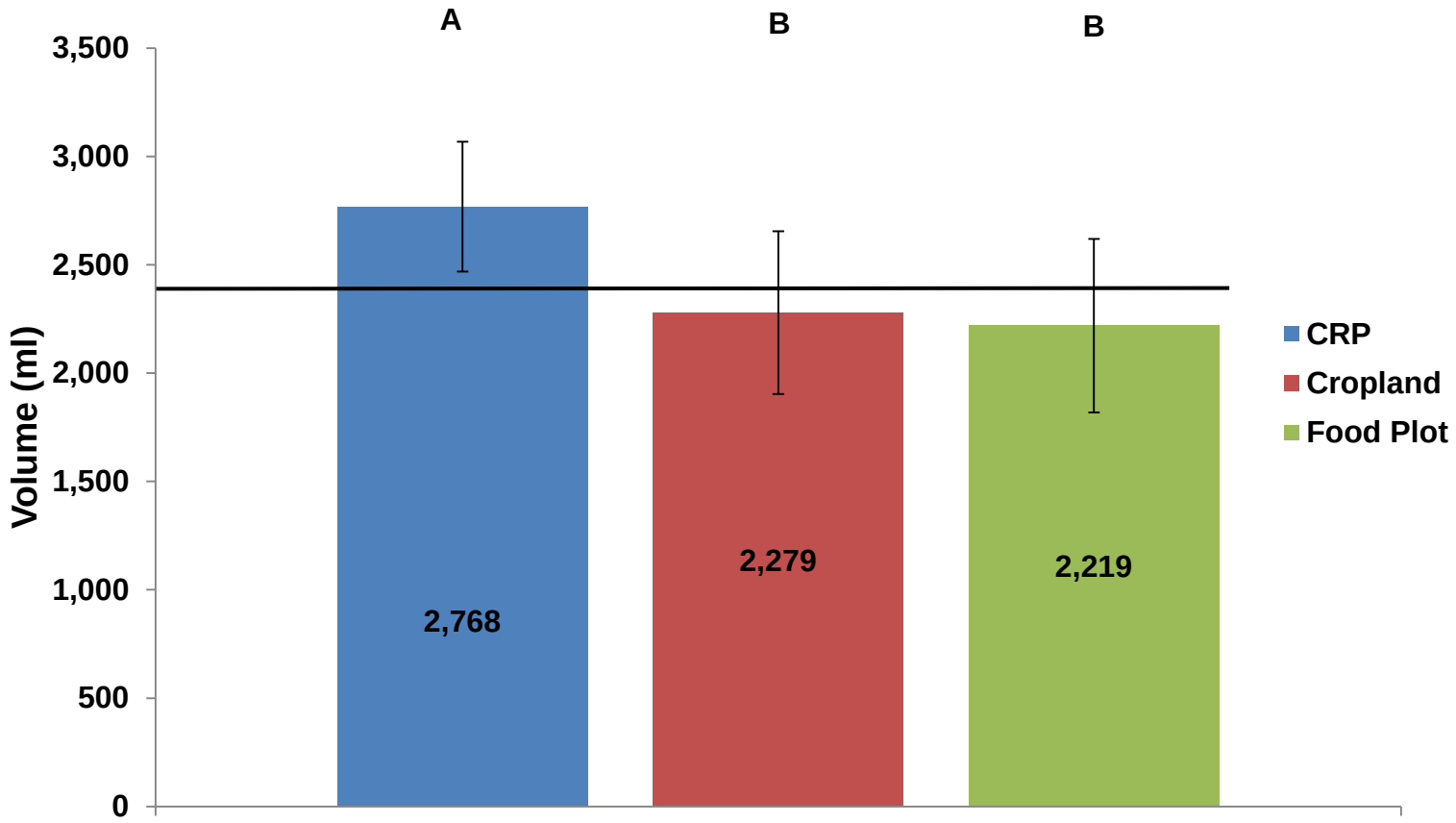

Figure 13. Mean soil sample volume ± Inter-quartile Range by landuse type from study site 2, Lyman County, South Dakota, 2012. Mean soil sample volume varied by landuse type (Kruskal-Wallis test, $P < 0.01$). Pairs with different letters were significantly different ($P < 0.05$) according to multiple tests for all-pairs comparison (Gao et al. 2008). The horizontal black line indicates the target soil sample volume of 2,419 ml according to a 30.5 X 61.0 X 1.3 cm sample.

Table 6. Comparison of negative binomial regression models for predicting spent lead shot availability within study site 3, Lyman County, South Dakota, 2013. Models are ranked by Akaike's Information Criterion adjusted for small sample size (AIC_c); K is the number of parameters, ΔAIC_c is the difference of each model's AICc value from that of the highest ranked model, and *ωi*, is the Akaike weight (sum of all weights = 1.00). All models except the intercept only model include the 2-level factor variable target shooting area.

Model	**K**	**AICc**	**∆AICc**	***ωi***
Distance walker * landuse type + distance blocker * landuse type	7	1107.15	0.00	0.84
Distance blocker + distance walker	4	1112.33	5.18	0.06
Distance blocker	3	1112.88	5.73	0.05
Distance blocker + landuse type	4	1113.96	6.81	0.03
Distance blocker + distance walker + landuse type	5	1114.28	7.13	0.02
Target shooting area	2	1140.75	33.60	0.00
Landuse type	3	1140.97	33.82	0.00
Distance walker	3	1142.75	35.60	0.00
Distance walker + landuse type	4	1142.77	35.62	0.00
Intercept only	1	1214.63	107.48	0.00

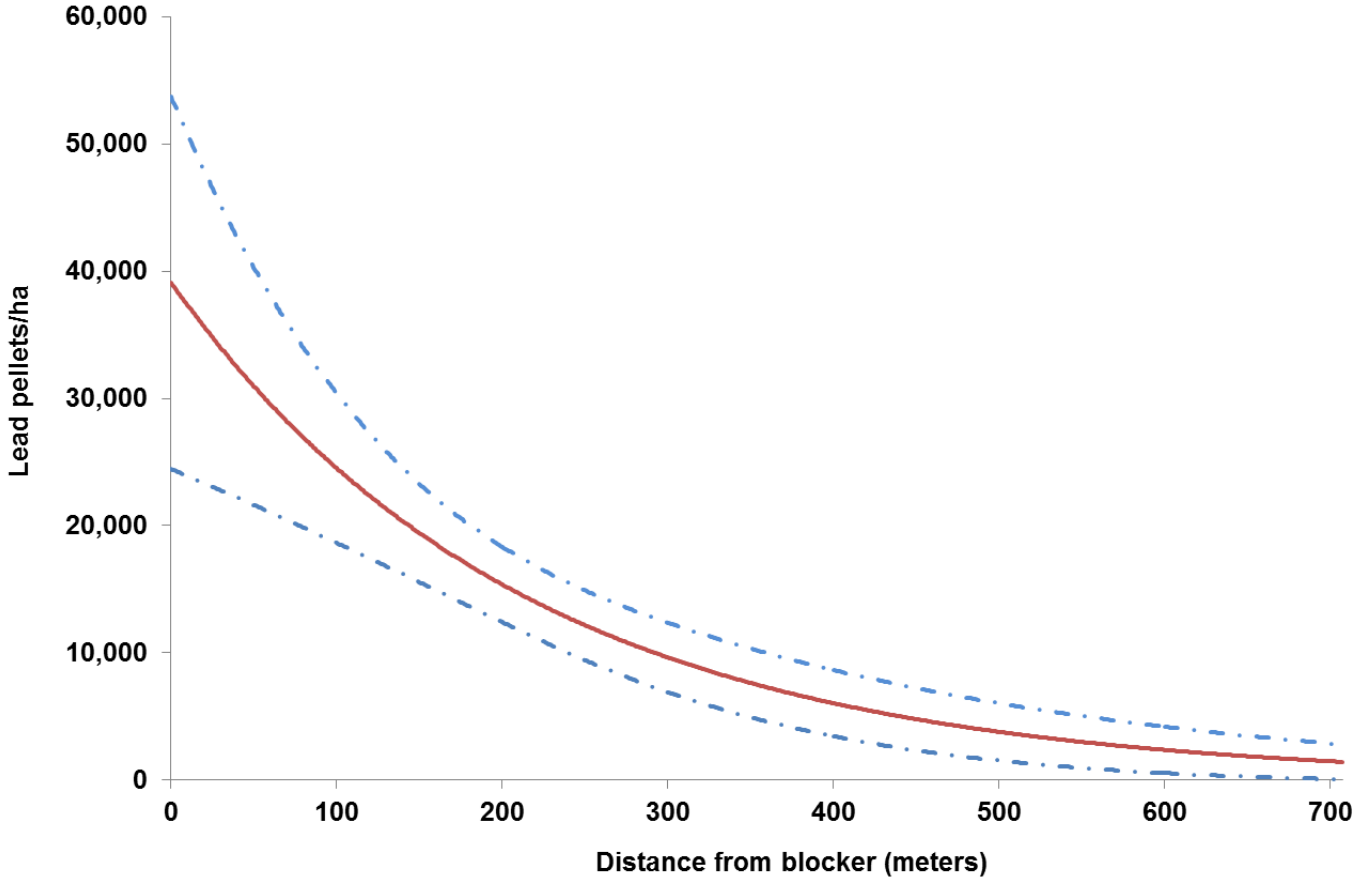

Figure 14. Predicted available lead pellets/ha ± 95% C.I. for study site 3, Lyman County, South Dakota, 2013. Predictions are based on the most parsimonious negative binomial model, (intercept + target shooting area + distance blocker). Predictions are for areas outside the target shooting area only.

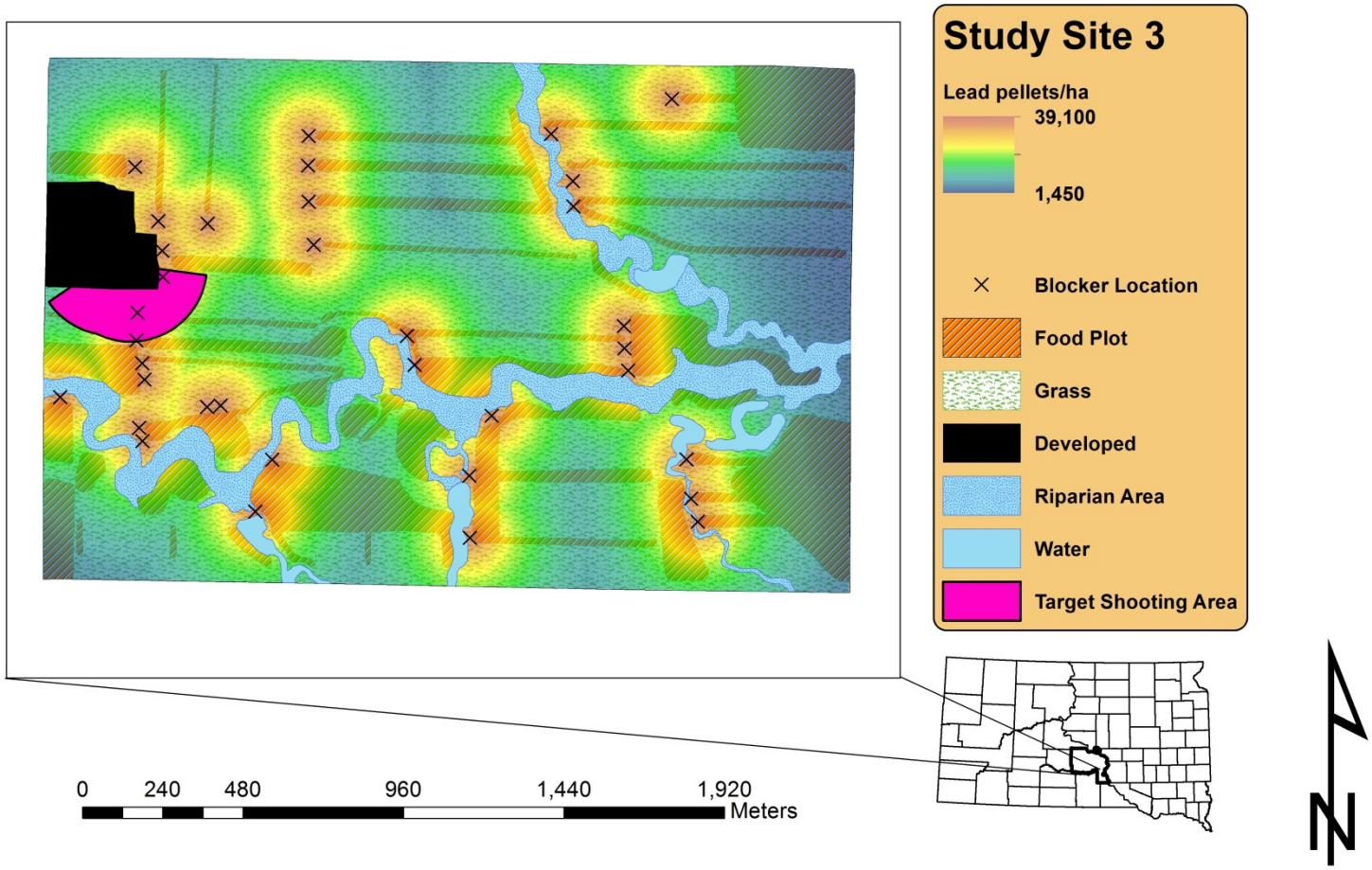

Figure 15. Predicted available lead pellets/ha for study site 3, Lyman County, South Dakota, 2013. Predictions are based on the most parsimonious negative binomial model as applied to areas outside of the target shooting area, (intercept + target shooting area + distance blocker).

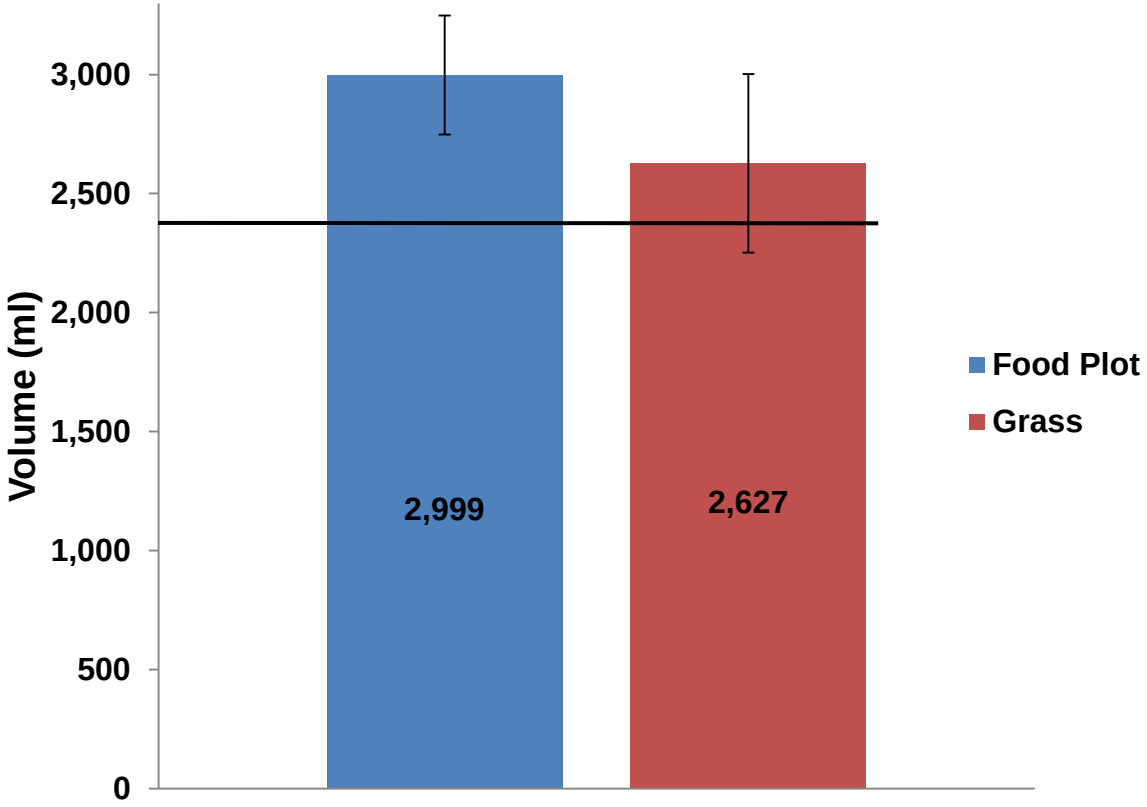

Figure 16. Mean volume ± Inter-quartile Range of soil sample by landuse type from study site 3 in Lyman County, South Dakota during sampling in July 2012. Mean soil sample volume varied by landuse type (Mann-Whitney U test, $P < 0.01$). The horizontal black line indicates the target soil sample volume of 2,419 ml according to a 30.5 X 61.0 X 1.3 cm sample.

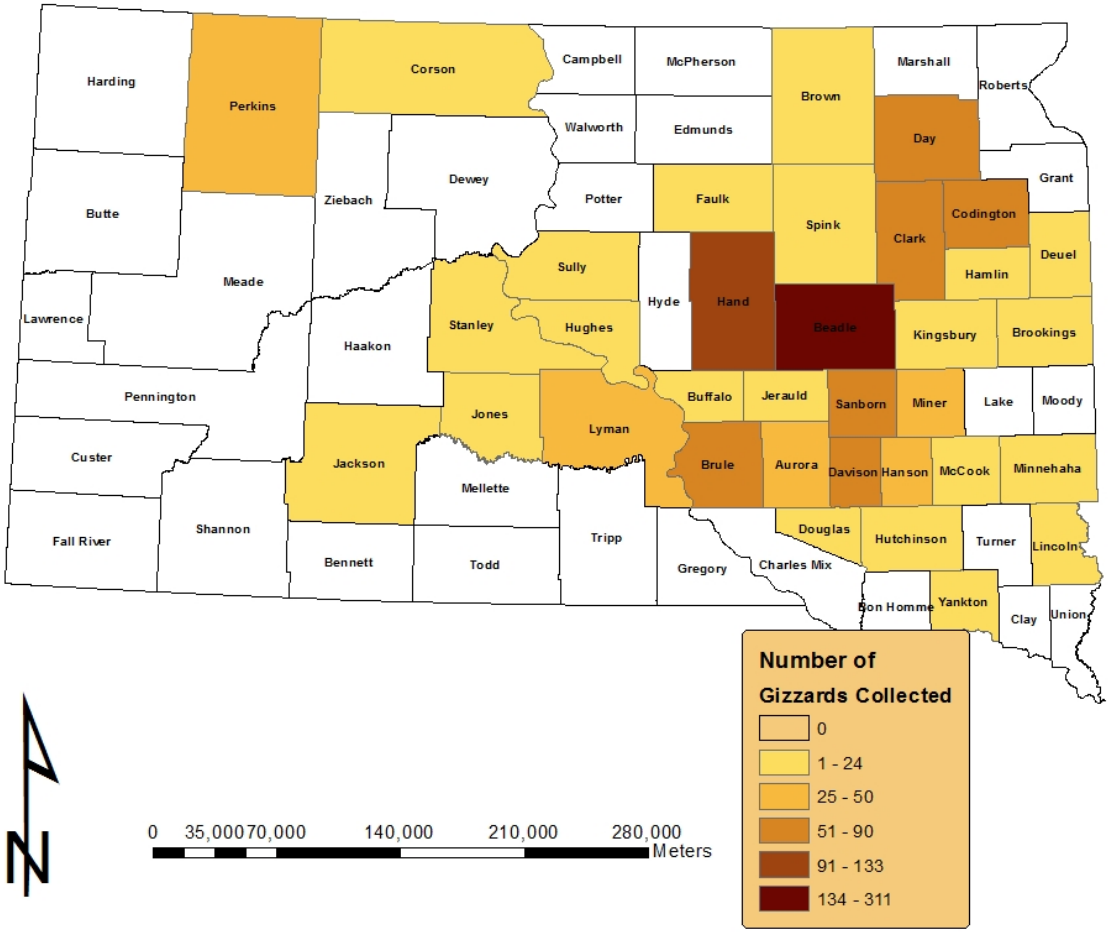

Figure 17. Map of the number of gizzards collected from hunter harvested wild ring-necked pheasants throughout South Dakota, 2013.

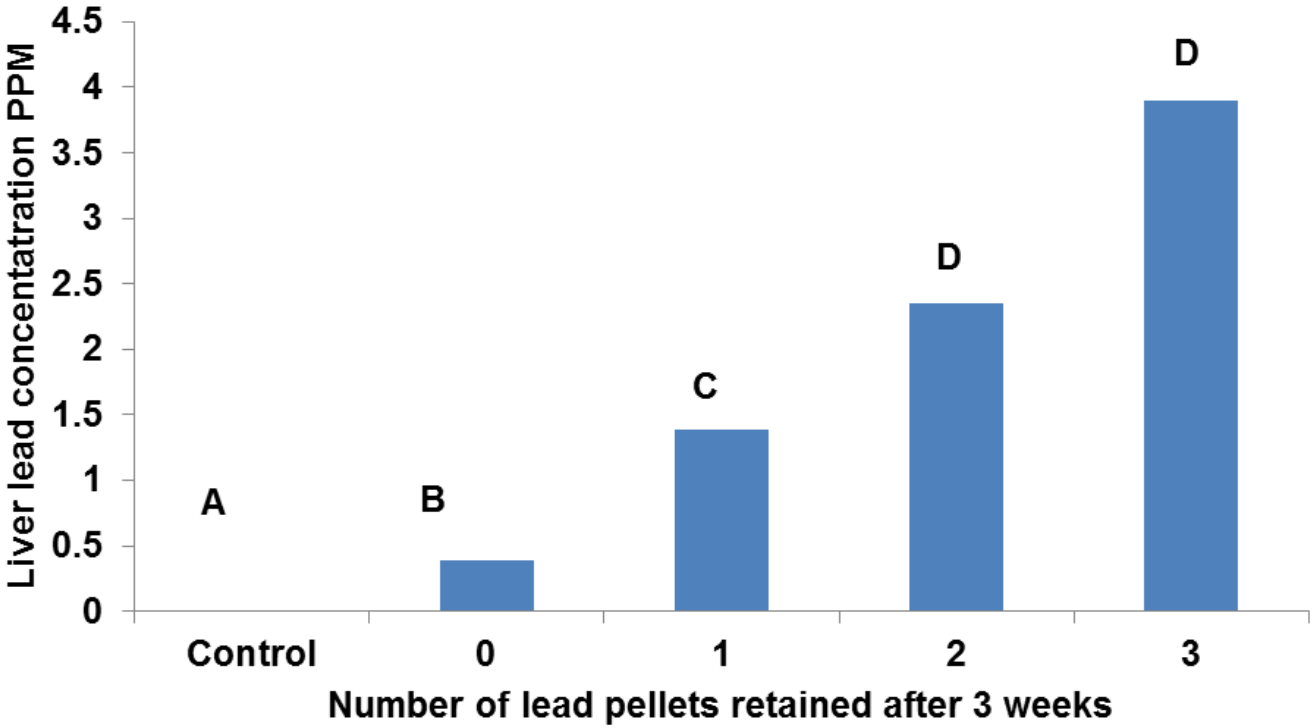

Figure 18. Liver lead concentration in response to the number of lead pellets retained 4 weeks after lead pellets were gavage-fed to captive hen pheasants. The mean liver lead concentration for pheasants in the control group was 0.00. Mean liver lead concentration varied by the number of lead pellets retained at the end of the 4-week trial (Kruskal-Wallis test, $P < 0.01$). Pairs with different letters were significantly different ($P < 0.05$) according to multiple tests for all-pairs comparison (Gao et al. 2008).

Table 7. Lead shot concentrations in upland habitats, including number of fields sampled, dimensions of each sample, volume of each sample, number of samples, number of lead pellets found and estimated density of lead pellets/ha for each study. A study by Holdner et al. (2004) was conducted on an island used heavily for put and take hunting and target shooting. This study was conducted on properties used for commercial pheasant hunting. All other studies were for managed dove fields.

Study	Number of fields sampled	Soil sample dimensions	Volume of soil collected per sample based on sample dimensions (cm^3)	Number of soil samples collected	Number of lead pellets found	Estimate of lead pellets/ha
Lewis and Legler (1968)	1	30.5 cm X 30.5 cm X 0.95 cm	883.74	100	81	27,132 - 107,639
Anderson (1968)	1	30.5 cm X 30.5 cm X 1.3 cm	1,209.34	150	84	23,500 - 73,200
Castrale (1989)	15	30.5 cm X 30.5 cm X 1.3 cm	1,209.34	2,406	Not Reported	0 - 83,928
Best et al. (1992)	1	30.5 cm X 30.5 cm X 1.3 cm	1,209.34	240	1,110	167,593 - 860,185
Schulz et al. (2002)[a]	2	30.5 cm X 30.5 cm X 1.0 cm	930.25	834	19	353 - 6,342
Holdner et al. (2004)	5	25 cm X 25 cm X 10 cm	6250	14	Not Reported	0 - 205,100,000
Douglas (2011)[b]	5	30.5 cm X 30.5 cm X 1.3 cm	1,209.34	4,204	2,654	67,813
This study - Site 1[a]	1	30.5 cm X 61 cm X 1.3 cm	2,418.68	640	236	1,186 - 166,172
This study - Site 2[a]	1	30.5 cm X 61 cm X 1.3 cm	2,418.68	746	107	2,300 - 11,000
This study - Site 3[a]	1	30.5 cm X 61 cm X 1.3 cm	2,418.68	685	413	1,450 - 625,000

[a]Mean actual soil volume per sample reported

[b] Mean pellet density reported. Pellet density varied with time and by crop type

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