Wildlife Research Final Report

Using DNA to Test the Utility of Pellet-group Counts as Indices of Deer Density

Todd J. Brinkman, David K. Person, Winston P. Smith, F. Stuart Chapin III, Karin R. McCoy, Matthew Leonawicz, and Kris J. Hundertmark

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Cover Photo: Sitka black-tailed deer adult doe and fawn on Prince of Wales Island; collecting collecting deer pellets for DNA extraction. Photos by and courtesy of Todd Brinkman, UAF

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ABSTRACT

Despite wide use of fecal pellet-group (PG) counts as an index of ungulate abundance and density, the utility of PG counts continues to be limited by variation in pellet detectability, persistence, and deposition rates. Moreover, techniques used to convert pellet densities to ungulate numbers are rarely based on counts of known individuals, seldom evaluated across spatial scales, and infrequently quantify precision. We tested the long-standing hypothesis that a linear relationship exists between PG density and ungulate density using a wild, abundant, and unenclosed population of Sitka black-tailed deer (SBTD) (*Odocoileus hemionus sitkensis*) in Alaska, USA. We simultaneously conducted PG counts and estimated deer density using DNA extracted from fecal pellets. Further, we evaluated performance of different PG survey techniques at multiple spatial scales, and used a bootstrap resampling method to identify how precision of the index changes with sampling intensity. From 2006–2008, we surveyed 141,054 m^2 of transect, counted 12,071 pellet groups, and identified 737 unique deer using DNA. Regardless of PG survey technique, PG density was not correlated with deer density at the watershed scale. At the individual transect scale, PG density was significantly correlated with deer density, but only using certain survey techniques. Using the linear regression equation derived from data with our best correlation, we estimated that 0.10 PG/m^2 equals 13.9 deer/km of transect (±3.5 deer [$\alpha = 0.10$]). Precision of deer density estimates based on PG densities expanded from ±25% to ±89% ($\alpha = 0.10$) when sampling intensity was reduced to match the protocol used to monitor SBTD in Alaska. Our results suggest that PG counts have utility to index deer numbers, but only with intensive sampling and at small spatial scales (i.e., <patch scale). We discourage extrapolation to larger scales (e.g., watershed) without additional testing of the influence of factors adding variability to PG counts. We recommend that ungulate monitoring programs periodically test PG count indices scales with independent data derived from known individuals. If feasible, we suggest that wildlife managers consider switching from PG counts to less variable and more reliable methods such as DNA-based approaches.

Key-words: Alaska, fecal pellet-group surveys, genetics, *Odocoileus hemionus sitkensis*, Sitka black-tailed deer.

INTRODUCTION

For over a half-century, monitoring programs of wildlife around the world have relied on fecal pellet-group (PG) counts to estimate size, trends, distribution, and habitat use of ungulate populations (Bennett et al. 1940, Rogers et al. 1958, Neff 1968 [comprehensive review], Baily & Putman 1981, Kirchhoff & Pitcher 1988, Koster & Hart 1988, Patterson & Power 2002, van Vliet et al. 2008). In many cases, PG counts were used because ungulate populations were living in densely forested environments and were difficult to monitor using other techniques requiring direct observation or live capture (Putman 1984, Ratcliffe 1987, Forsyth et al. 2007, van Vliet et al. 2008).

Most studies that have used PG counts as an index have evaluated the performance of the technique to track the population parameter of interest. Despite wide use and rigorous evaluation, the value of PG counts as an index of wildlife numbers and population change continues to be a contentious issue. Some authors reported that PG counts index ungulate abundance well (Forsyth et al. 2007, Acevedo et al. 2010) whereas others suggested the value of PG counts as an index lacks utility and reliability (Ryel 1971, Fuller 1991, Campbell et al. 2004, Smart et al. 2004).

Factors limiting the use of PG counts as an index of population trends include human error (e.g., pellet detectability, observer experience), variation in pellet deposition rates and pellet persistence (e.g., influence of weather, insects), and the lack of uniformity in PG distribution (Neff 1968, Jenkins & Manly 2008). Moreover, in many circumstances, procedures to convert PG counts to numbers of animals are based on few empirical data, seldom evaluated over time, and precision associated with estimates rarely quantified. Given the potential for combinations of those factors to confound or mask relations between PG counts and actual populations, researchers have sought alternative strategies to monitor ungulates.

Over the last decade, development of genetic techniques using non-invasive sampling has advanced opportunities to individually identify rare and elusive forest-dwelling wildlife (Waits & Paetkau 2005, Schwartz & Monfort 2008). In recent years, rapid expansion of genetic technologies has allowed researchers to monitor abundant populations of ungulates using DNA from hair and feces (Belant et al. 2007, Van Vliet et al. 2008, Grebremedhin et al. 2009). If an adequate number of individuals are identified, then mark-recapture estimators of population size are possible (Huggins 1991, White 2008, Kendall et al. 2008). More recently, Brinkman et al. (accepted) used DNA extracted from deer pellets to estimate abundance of a wild and unenclosed deer population with precision of ±20%.

We conducted PG counts and DNA-based methods to simultaneously estimate deer abundance in 3 watersheds in Southeast Alaska enabling us to compare results from both methods and to further explore the utility of PG counts. Motivated by an opportunity to improve a 30-year old monitoring program of Sitka black-tailed deer (SBTD) (*Odocoileus hemionus sitkensis*) in Southeast Alaska, our objective was to compare PG densities of SBTD with DNA-based estimates of deer density based on known individuals. Given the volume of literature on PG survey techniques, there are surprisingly few studies that compare PG counts with estimates of ungulate density derived from known individuals. In an attempt to further support or reject the

use of PG counts as a useful index of ungulate population size and trends, we test the long-standing hypothesis that a linear relationship exists between PG density and ungulate density in a wild, abundant, and unenclosed population of SBTD. We evaluate performance of different PG survey techniques at multiple spatial scales. Lastly, we used a bootstrap resampling method to identify how precision of our estimates of deer density changes with sampling intensity of PG counts.

In addition to the broader contribution of evaluating a technique used frequently around the world, our study also has immediate application and important regional implications. In Southeast Alaska, SBTD are a barometer of ecosystem health, a key indicator of the effects of forest management, and the most important terrestrial species for both sport and subsistence hunting (Hanley 1993, Brinkman et al. 2009). Over the last 3 decades, standardized PG counts have been used as the primary tool to monitor population trends of SBTD at large spatial and temporal scales (Kirchhoff & Pitcher 1988). Despite heavy reliance on these data, PG counts of SBTD have been compared to another independent measure of population size only once in Southeast Alaska (Kirchhoff 1990). Because of the assumptions and uncertainty associated with Kirchhoff's (1990) study, those data are considered insufficient for use as an index to estimate population size and monitor SBTD at scales useful for game management in Southeast Alaska (Unit 2 Deer Planning Subcommittee 2005).

During Kirchhoff's (1990) study, 13 radiocollared deer were transported to a small and isolated island (0.4 km^2) which, according to field reconnaissance prior to release, was not inhabited by other deer. Nine months after release, intensive PG counts (Kirchhoff 1990) were conducted on the island. During the 9 months prior to PG counts, 3 deer died and 6 deer swam off the island leaving a population of 4 deer on the island. Taking into account departure dates of each deer, the amount of deer use on the island was considered equivalent to 6 deer spending the entire period on the island (Kirchhoff 1990). Therefore, the relationship between PG density and deer density was derived using a population of 15 deer/km^2 over the study period (Kirchhoff 1990). Persistence and deposition rates of pellets were not directly monitored, and assumed to be similar among deer and over time.

The inability to reliably monitor deer abundance has thwarted efforts to address contentious issues related to deer hunting, and SBTD habitat in Southeast Alaska (Brinkman et al. 2009). During 2006–2008, we developed a mark-recapture method based on DNA extracted from fecal pellets and a survey design that samples pellets along deer trails to obtain more precise and reliable data on SBTD numbers. Using those methods, we were able to estimate deer abundance and density over a 3-year period with precision of ±20% (Brinkman et al. accepted). In this paper, we compare estimates of deer abundance from that study to estimates derived from pellet counts obtained from the same locations and during the same years. We address pellet persistence, on the key factors confounding interpretation of pellet counts and we employ a path sampling strategy that increases pellet detection and the number of pellet groups observed. We wanted to determine if traditional or improved methods of PG counts could produce reliable indices of deer abundance. Our DNA-based abundance estimates provided compelling evidence that the deer population in the study area declined owing to 3 consecutive severe winters. Those events enabled us to determine if pellet counts could detect and reliably monitor that trend. If a consistent relationship was identified between PG counts and DNA-based estimates of abundance, then deer populations could be cost-effectively estimated and monitored using PG

counts, which are calibrated or "truthed" periodically using DNA-based estimates as benchmarks.

METHODS

We conducted our research on Prince of Wales Island, which is located near the south end of the southeastern panhandle of Alaska (Fig. 1). The topography included rugged mountains extending to 1,160 m in elevation with landscapes below 600 m dominated by temperate coniferous rainforest consisting primarily of Sitka spruce (*Picea sitchensis*) and western hemlock (*Tsuga heterophylla*) (Alaback 1982). Annual precipitation varied from 130 to 400 cm, and mean monthly temperatures ranged from 1°C in January to 13°C in July. Between winters 1948–2008, mean annual snowfall at sea level was 115 cm (SE = 9.5) at the closest weather station on Annette Island (Alaska Climate Research Center 2009). Snowfall was above the mean in all sites during our study period, with 128 cm, 187 cm, and 161 cm of snowfall during 2006, 2007, and 2008, respectively. Within each study site, elevation extended from 0–1000m. Snowfall, snow depth, and persistence increased with elevation.

We established study sites in the Maybeso Creek (Maybeso), upper Staney Creek (Staney), and upper Steelhead Creek (Steelhead) watersheds located within the north-central portion of Prince of Wales Island (Fig. 1). All study sites were accessible by road and deer are actively hunted from August until January. Each study site encompassed a mosaic of productive old-growth forest, unproductive forests on hydric soils, open muskeg heaths, and clearcut forest at various successional stages (logged 5–60 years ago). Other mammals (e.g., wolves [*Canis lupus*], black bears [*Ursus americanus*]) occurred within the study area, but none with fecal deposits that could be confused for deer pellets.

We conducted PG counts in each watershed using traditional straight-line transects (Kirchhoff & Pitcher 1988) and trail transects (Brinkman *et al.* accepted). Traditional straight-line transects are currently incorporated into the SBTD monitoring program and have been used by wildlife agencies in Southeast Alaska for 3 decades. Trail transects were designed to facilitate mark-recapture methods to estimate abundance of deer using DNA from fecal pellets (Brinkman *et al. accepted*).

Following Kirchhoff & Pitcher (1988), traditional transects consisted of a series of contiguous 1x20 m plots extending from a predetermined starting point and continuing until a predetermined distance or elevation was reached. Traditional transects were mainly established in productive old-growth forest, considered critical winter habitat for deer (Schoen & Kirchhoff 1990). Other major habitat types (e.g., clearcut forest, muskeg, second-growth forest) were not included in the survey design. Transects were typically surveyed by a team of two people following a preselected compass bearing. The lead team member pulled a 20-m plastic-coated steel cable and stopped at 20-m intervals. The second team member counted all pellet groups within 0.5 m of either side of the cable. All pellet groups, regardless of age were counted. Traditional transects were surveyed once a year. Pellet density was calculated by dividing the total area of the transect by the number of PG counted. Under the current SBTD monitoring program, researchers survey approximately 5,000 m^2 in selected watersheds (McCoy 2008). Ideally, that sampling intensity should enable an estimation of the mean number of pellet groups per transect per watershed within 15–25% of the true value ($\alpha = 0.05$) (Kirchhoff & Pitcher 1988).

Trail transects followed deer trails through the forest. They were established in all major habitat types: productive old-growth forest, unproductive forests on hydric soils, open muskeg heaths, and clearcut forest at various successional stages (logged 5–60 years ago). Similar to a straight-line transect, we had a predetermined starting point and survey direction with systematic sampling. We traveled in the direction of a predetermined bearing from the starting point until a deer trail was encountered. The deer trail was followed in the direction closest to the bearing until intersected by another trail. We then used a compass to determine which trail more closely paralleled the direction of the predetermined bearing and continued surveying along that trail. If the trail ended or a trail could no longer be identified, we followed a straight-line path along our bearing until another deer trail was encountered. The lead team member would use the compass to select the deer trail to be surveyed. The second team member would follow closely behind, counting all pellet groups, and intensively marking the trail being surveyed. Because of greater visibility of the forest floor along deer trails, all pellets within 1 meter of the center of the trail were counted. Pellet density was calculated by dividing the total area of trail transect by the number of pellet groups counted. Further details concerning our trail transect design and assumptions associated with this survey technique can be found in Brinkman *et al.* (accepted) and Brinkman (2009).

Brinkman (2009) found that trail transects had several advantages over traditional straight-line transects, including: higher encounter rates with pellet groups (48% higher than straight-line transects), applicability in all habitat types, better pellet-detection rates, easier travel through thickly-vegetated habitats, and greater repeatability. Fundamentally, the trail transect focuses sampling along trails where activity of deer is greater compared to randomly located straight-line transects.

We used both methods (traditional and trail transects) to survey for pellets simultaneously, and all traditional transects overlapped the same forest patches as a trail transects. A trail transect was established to have approximately the same starting point and traversed through the same habitat patch(es). Differences in distance between overlapping traditional and trail transects were accounted for by converting PG counts to PG density (PG/m^2). Therefore, the effects of observer, habitat, pellet deposition rates, pellet persistence, weather, and deer distribution and abundance were assumed to be similar between the 2 methods. For both methods, a pellet group was defined as several pellets of the same size, color, and shape that were positioned in a clumped distribution.

We collected pellets for DNA analysis while conducting PG surveys. We followed sampling, DNA extraction, genotyping, analysis, and abundance estimation protocols described in Brinkman *et al.* (*accepted*, 2010a). Briefly, we re-sampled path transects 2–8 times per annual field season in 10-day intervals. Number of sampling occasions was weather dependent. We collected 4–6 pellets from each pellet group encountered within the prescribed 2-m wide sampling area of the transect. After recording location and time for each pellet group sampled, all pellets were removed from the transect to avoid re-sampling from the same pellet group during subsequent sampling occasions. Because the time interval since deposition was unknown on pellet groups encountered during the first sampling occasion, we collected only from pellet groups with characteristics of recent deposition (freshly deposited in a clumped distribution with pellets intact, surface with a glossy sheen, and a detectable coating of mucus) (Brinkman *et al.* 2010a, b). We sampled fewer pellet groups that appeared in "poor" condition during the first

sampling occasion during the 2008 field season to reduce the risk of processing samples with insufficient DNA to genotype a deer. All pellet groups encountered during subsequent surveys were sampled except those exposed to severe weathering (e.g., pellets deposited in standing water) between sampling occasions, which represented approximately 7.5% of all pellet groups encountered during subsequent sampling occasions. Individual deer were identified using DNA extracted from fecal pellets collected from pellet groups encountered along trail transects (Brinkman *et al.* 2010a)

Total counts of all pellet groups encountered during each annual field season allowed calculation of a 'standing crop' of pellet groups (Staines & Ratcliffe 1987). Clearing all pellets during the first sampling occasion and re-sampling several times each field season allowed us to also count the 'clearance crops' of pellet groups (Staines & Ratcliffe 1987, Campbell *et al.* 2004, Smart *et al.* 2004). Standing crop represented the sum of pellet groups that accumulated over winter on our transects, plus the pellet groups deposited during our annual field season. Clearance crop represented only pellet groups encountered after the first sampling occasion. Clearance PG counts reduced the confounding of pellet persistence (Harestad & Bunnell 1987, Jenkins & Manly 2008). Clearance PG counts still suffer from biases associated with variability in defecation rates, which may vary with time of year and individual (Mitchell *et al.* 1985, Harestad & Bunnell 1987). Nonetheless, because traditional transects, trail transects, and pellet sampling for DNA were performed simultaneously and within the same habitat patches, defecation rate likely was not a confounding factor with respect to comparing methods.

We converted PG counts to PG density (PG/m^2 of transect) for both traditional and trail transects. We converted the number of individual deer identified to relative deer density (deer/km of transect). We compared the relationship between PG density (PG/m^2) and deer density (deer/km of transect) for traditional and trail transects in 3 different ways at both the watershed and individual transect spatial scale within each study site during each annual field season:

1) We compared PG densities on traditional transects with deer density on trail transects that overlapped traditional transects (hereafter, these trail transects will be referred to as "overlapping transects"). Because traditional transects were surveyed once a year, only standing crop could be evaluated.
2) We compared both standing crop and clearance crop PG densities along trail transects with deer density along trail transects.
3) With traditional PG counts in Alaska thought to be representative of deer activity in a watershed (Kirchhoff & Pitcher 1988), we compared PG densities on both traditional and trail transects with abundance estimates reported by Brinkman *et al.* (accepted) in the same watersheds during the same year. Those abundance estimates were derived from a DNA-based mark and recapture study.

Data were coded and analyzed using the statistical computer programs SPSS 12.0.1 (SPSS Inc., Chicago, Illinois, USA) and R (R Development Core Team 2009). We used a nonparametric chi-squared test (χ^2) to compare DNA-based estimates among individual transects, study sites, and years ($\alpha = 0.05$). We measured association between variables using Pearson's correlation coefficient. We fit lines to scatterplots to illustrate linear relationships between variables and reported corresponding regression equations and R^2 values.

To measure how precision of deer density estimates changed as a linear function of PG densities at varying sampling intensities, we used a bootstrap re-sampling method. To correct for heteroskedasticity (i.e., non-constant variance) in PG densities on individual transects, we performed a weighted (generalized) least squares (WLS) regression on our data. We estimated mean response from the linear regression equation (deer density = slope × PG density + intercept) at varying confidence levels ($\alpha = 0.05, 0.10, 0.15$) at the following sampling intensities: using all transects (i.e., maximum intensity), 75 transects (i.e., high intensity), 50 transects (i.e., medium intensity), 25 transects (i.e., low intensity), and 12 transects (i.e., minimal intensity). The "minimal intensity" category matched the current sampling intensity (~5000 m^2 of area surveyed per watershed) of the SBTD monitoring program (McCoy 2008). At each of our sampling intensities, we used bootstrap resampling to obtain 10,000 random samples of transects. For each of these samples we fit a WLS regression. From these data we derived bootstrapped confidence intervals corresponding to each sampling intensity and confidence level. To calculate the upper and lower bound on the mean response at each confidence interval, we used the corresponding bootstrapped standard error for slope and intercept. Specifically, we first obtain a point estimate for the mean response using the original (maximum intensity-based) weighted least regression equation. Then for each confidence level, a confidence interval centered on this estimate is constructed in the standard way using a *t*-distribution. However, the standard error of the bootstrapped slope and intercept corresponding to each sampling intensity are used in place of the original slope and intercept standard errors, respectively.

RESULTS

PELLET-GROUP COUNTS

From 2006 through 2008, we conducted PG counts along traditional straight-line ($n = 24$ [8 per year]) and trail ($n = 93$ [31 per year]) transects in Maybeso, Staney, and Steelhead watersheds (Table 1). We surveyed traditional transects once each year during early May and we surveyed trail transects a mean of 5.0 (SE = 0.12) times each year during the months of March through May. All traditional transects, except for 1 in Staney, overlapped with a trail transect. We counted 1,502 pellet groups along traditional transects, 10,569 pellet groups along trail transects, and 3,708 pellet groups along trail transects that overlapped (i.e., overlapping transects) traditional transects. Of all the pellet groups encountered along the multiple surveys conducted each year on trail transects, 77% were encountered during the first survey of each annual field season (i.e., standing crop), which represented over-winter deposition. Therefore, the PG count after clearing (i.e., clearance crop) was 2,445 on trail transects and 771 on overlapping transects.

Mean standing PG densities (pellets/m^2) at the individual transect scale were 0.098 (SE = 0.011, range = 0.209), 0.096 (SE = 0.006, range = 0.331), and 0.089 (SE = 0.007, range = 0.140) on traditional, trail, and overlapping transects, respectively (Table 2). Mean clearance crop PG density at the individual transect scale was 0.023 (SE = 0.002, range = 0.133) and 0.020 (SE = 0.004, range = 0.078) on trail and overlapping transects, respectively. At the watershed scale, we identified a positive, but non-significant (Pearson = 0.407, $P = 0.318$) relationship between standing PG densities on traditional transects and overlapping transects (Fig. 2). At the individual transect scale, standing PG densities were not correlated with overlapping trail transects (Pearson = 0.095, $P = 0.660$) (Fig. 2). Standing PG densities were uncorrelated with

clearance PG densities on trail transects at the watershed scale (Pearson = 0.139, $P = 0.721$), but correlated at the scale of an individual transect (Pearson = 0.567, $P = <0.001$) (Fig. 3).

DNA-BASED ESTIMATES

During the months of March through May of 2006 through 2008, we collected 2,248 fecal-pellet samples for DNA analysis, successfully genotyped 1,156 (51%) samples, and identified 737 unique deer. Genotyping success on individual transects was similar among study sites ($\chi^2 = 0.136$, $P = 0.934$), but different across annual field seasons ($\chi^2 = 48.14$, $P = < 0.001$). During 2008, 87% of pellet groups sampled yielded sufficient DNA to genotype an individual deer, which was a result of fewer poor-quality samples being collected during the first survey. Combining all years, 17.7% of the pellet groups encountered during the first survey of trail transects were sampled. Proportion of pellet groups sampled on individual transects during first sampling occasion was similar among study sites ($\chi^2 = 2.298$, $P = 0.317$) within years, but different across years ($\chi^2 = 2.298$, $P < 0.001$). We sampled a greater proportion of pellet groups encountered during first sampling occasions in 2006 and 2007; however, opportunities to identify deer along transects were adjusted during DNA analysis because the proportion of pellet groups that failed to amplify during 2006 and 2007 was equal to the differences in sampling effort. During 2006, 2007, and 2008, approximately 30%, 45%, and 91%, respectively, of pellets collected during the first sampling occasion on trail transects yielded adequate DNA to successfully genotype a deer. However, the percentage of samples genotyped during the first sampling occasion, relative to the number of pellet groups encountered during the first sampling occasion, was nearly identical during 2006 (5.7%), 2007 (6.0%), and 2008 (4.6%), despite great differences in proportion sampled.

The number of genetically unique deer identified along trail transects was highest in 2006 and declined in all study sites during the 3-year study when all trail transects were included in analysis and when only trail transects that overlapped with traditional transects were included (Table 3). We estimated the greatest decline in deer density in Maybeso (44%) when all transects were included in the analysis, and we estimated the greatest decline in deer density in Staney (69%) when only overlapping transects were included (Table 3).

STANDING CROP AND DEER DENSITY

At the watershed scale, we determined that standing PG densities on traditional ($n = 8$, Pearson = -0.400, $P = 0.326$), trail ($n = 9$, Pearson = -0.277, $P = 0.556$), and overlapping transects ($n = 9$, Pearson = 0.218, $P = 0.573$) were not correlated with deer densities (Fig. 4). In many cases, as standing PG densities (PG/m^2) increased, both DNA-based estimates of deer densities (deer/km of transect) and Brinkman et al.'s (accepted) estimates of deer abundance decreased (Fig. 4). For instance, at the watershed scale, we determined that standing PG densities on traditional transects (Pearson = -0.760, $P = 0.029$) were negatively correlated with Brinkman et al.'s (accepted) abundance estimates. Standing PG densities on trail (Pearson = -0.421, $P = 0.259$), and overlapping transects (Pearson = -0.079, $P = 0.841$) were not correlated with abundance estimates (Fig. 4).

When evaluating transects individually, we identified a weak negative but non-significant relationship between standing PG densities on traditional transects ($n = 24$) Pearson = -0.235, P

= 0.269) and deer density (Fig. 4). We identified significant positive relationships between deer densities and standing PG densities on both trail (n = 93, Pearson = 0.579, P = <0.001) and overlapping (n = 27, Pearson = 0.450, P = 0.018) transects (Fig. 5).

CLEARANCE CROP AND DEER DENSITY

At the watershed scale, we determined that clearance PG densities on trail (Pearson = 0.055, P = 0.888), and overlapping transects (Pearson = 0.573, P = 0.218) were uncorrelated with deer density (Fig. 5). We also determined at the watershed scale that clearance PG densities on trail (Pearson = -0.044, P = 0.911), and overlapping trail transects (Pearson = 0.206, P = 0.594) were uncorrelated with abundance estimates (Fig. 5). When evaluating transects individually, we identified a positive and significant correlation between clearance PG densities on trails (Pearson = 0.489, P <0.001) and deer densities, and with clearance PG densities on overlapping transects (Pearson = 0.440, P <0.001) and relative deer densities (Fig. 5).

PRECISION OF DEER DENSITY ESTIMATES USING PELLET COUNTS

Because we determined that standing crop PG densities at an individual trail transect scale had the most highly correlated relationship with deer density (Fig. 4C3), we performed our test of precision at varying sampling intensities using the WLS regression equation this scale (deer/km of transect = 1.615 + 122.897 × PG density, R^2 = 0.50). Using the mean PG density (n = 93) along trail transects (0.10 PG/m^2) (i.e., predictor value), our deer density estimate (i.e., response value) was 13.9 deer/km of transect (Table 4). Using α = 0.10, precision of deer density predicted using the mean PG density along trail transects expanded from ±25% using the maximum sampling intensity (93 transects) to ±89% using the minimum sampling intensity (12 transects) (Table 4). The lack of a linear relationship using traditional transects did not allow a calculation of precision using those data.

DISCUSSION

Recent PG count studies have continued to focus on strategies to quantify or limit the variability introduced by factors (e.g., persistence, observer error) (Jenkins & Manley 2008), determine whether return justifies effort (Campbell *et al.* 2004), and to compare the performance with alternatives (Acevedo *et al.* 2010). We have added to this discussion by comparing PG counts with a new alternative based on genetically identified individual deer (Brinkman *et al.* accepted). Despite studies (e.g., Forsyth *et al.* 2007) that report PG counts as useful indices of ungulate abundance when comparing against known numbers, it is evident that the utility of this index varies with research location, species, and approach. We found a weak association (and sometimes misleading) between the number of pellet groups and number of deer.

SCALE

Neither traditional nor trail transects were associated with deer abundance or density when pooling transects and evaluating at the watershed scale. We did find a relationship between PG densities on trail transects and deer densities, but only at the individual transect level. Our results suggest that PG counts have utility to index deer numbers within areas immediately

adjacent to transects, but extrapolation to larger scales (e.g., watershed) should be discouraged. As transects were pooled, the linear relationship became less clear. Apparently, PG densities have a different relationship with deer density at different scales of evaluation because of variability among individual transects in the relationship between PG density and deer density. This variability likely resulted from landscape heterogeneity increasing as the area surveyed expanded. A single transect undoubtedly traverses a less heterogeneous landscape than all transects in the watershed combined. For instance, Sitka black-tailed deer select for and avoid certain habitats and landscape features (Schoen & Kirchhoff 1990, Doerr *et al.* 2005). We speculate that the patchy use of the environment leads to variability in the relationship between PG density and deer density. Further, deer behavior, such as foraging and travel rates, likely varies among habitat types and contributes additional variation associated with landscape heterogeneity.

TRADITIONAL TRANSECTS VS. TRAIL TRANSECTS

We determined that PG counts using trail transects had more utility as an index of deer population size than PG counts using traditional transects. Indeed, traditional transect counts and deer density (Fig. 4 B1) were negatively related if at all. The lack of a relationship between traditional transects and overlapping transects (Fig. 2) suggests that traditional transects also may not be representative of deer numbers within the same patch that the transect traverses; whereas, an association was evident for overlapping transects surveyed at the same intensity (Fig. 4 C1, C2). We speculate that trail transects may have performed better than traditional for transects for 3 reasons. First, the DNA used to identify deer came from pellets collected from trail transects and not from traditional transects. Second, we speculate that observer bias (Neff 1968, Jenkins & Manly 2008) was lower on trails compared to straight-line transects because of a more visible forest floor along deer trails. Thirdly, both sample size (i.e., # of transects) and opportunities to encounter pellet groups per distance surveyed was greater on trail transects, which potentially improved statistical inference. Although we identified higher PG densities on traditional transects (0.95) compared to trail transects (0.87), that result may be misleading. The lower PG densities on trail transects likely was because both good deer habitat and poor deer habitat were surveyed during trail transects. Whereas, traditional transects were established in areas considered critical winter habitat for deer (Schoen & Kirchhoff 1990). For example, a 1-km trail transect that traversed through the middle of an open muskeg had PG densities below 0.02 each year of the study. Further, the high variability in PG densities using traditional transects allow abnormally high or low numbers just by chance. Lastly, the expanded sampling area on trail (2-m strips) transects also lowered overall PG densities. When only 1-m strips were surveyed on both traditional and trail, Brinkman (2009) determined that PG densities were 48% greater on trails. The majority (~82%) of pellets deposited on trail transects were within 0.5 meters of the center of the trail. The 1 meter buffer was chosen on trail transects to maximize opportunities to encounter DNA per unit effort.

STANDING VERSUS CLEARANCE CROP

Although the scale of evaluation influenced the relationship between PG density and deer density, the collection strategy (standing crop vs. clearance crop) did not. In theory, clearance crop collection eliminates assumptions associated with pellet persistence (Staines & Ratcliffe 1987), and has been shown to increase precision of variable estimates (defecation rate, PG

counts, and decomposition rate) used for abundance estimates if direct counts of deer are unavailable (Campbell 2004). In practice, we were unable to locate a study reporting that clearance plots significantly increase the utility of PG counts as an index of ungulate population size. Smart *et al.* (2004) determined that clearance crop approaches performed poorly compared to standing crop and could not recommend continued use. However, Campbell *et al.* (2004) suggested that Smart *et al*'s (2004) findings may be due to the time interval between sampling occasions. Campbell *et al.* (2004) found a small but consistent difference in precision, favoring clearance crop. During our study, 'clearing' removed over-winter deposition, but the variability reduced by eliminating the effects of deposition rate may have been negated by the increased variability from smaller sample sizes (i.e., pellet groups encountered) that were typical of clearance crops.

PRECISION

The survey intensity of the traditional transects was approximately 40% of the typical sampling intensity applied per watershed in Southeast Alaska (McCoy 2008). According to a power analysis performed by Kirchhoff & Pitcher (1988), our PG densities, at our sampling intensity, should be within 25% of the mean PG density 95% of the time at moderate deer densities (0.05–0.1 PG/m^2). However, even if those confidence intervals were applied to PG densities on traditional transects (Fig. 4), positive linear relationships would not be possible.

Because Kirchhoff & Pitcher's (1988) precision estimates reflected variation between PG densities on transects (not estimates of deer density), our results are not directly comparable with theirs. We were interested in the utility of PG densities as an index of deer density and determined a precision of ±115% (using data with the best linear relationship [93-transect WLS regression equation]) at a sampling intensity and confidence interval used by the SBTD monitoring program. At that precision and at a sampling intensity that is triple the current level employed by the SBTD monitoring program, actual deer density would have to change by >50% before researchers could detect a statistically significant change ($\alpha = 0.10$) using PG densities (Table 4).

MANAGEMENT IMPLICATIONS

We suggest that PG surveys done in a manner similar to those in Southeast Alaska are unreliable indices of deer population size and trends. Although some studies support the use of PG counts for indexing deer abundance (Forsyth *et al.* 2007, Acevedo *et al.* 2010), those studies were conducted under conditions in which many of the confounding factors associated with pellet-group surveys were controlled and with sampling intensity not likely to be repeated at large scales. For instance, Forsyth *et al.* (2007) validated the utility of PG counts with known numbers of deer in intensively-managed hunting enclosures in New Zealand. Acevedo *et al.* (2010) also conducted their study in an intensively managed area (e.g., artificial feeding, water provisioning) in Mediterranean habitat in Spain, which could be considered dry and open relative to a coastal temperate rainforest. Accessibility of survey areas, ruggedness of terrain, density of understory vegetation, weather severity are all factors that limit the feasibility of using intensive pellet counts as population indices other than for small confined populations. We suggest that monitoring programs using pellet counts consider our trail sampling protocol, sample all available habitats, and periodically test trends and estimates with independent data based on known individuals, such as DNA-based method or direct observation if possible. At the very

least, factors (e.g., habitat, season) influencing the relation between pellet counts and actual abundance should be investigated and modeled if possible so that the influence of those factors can be assessed when data from pellet count surveys are interpreted. If feasible, wildlife managers should consider switching entirely from pellet counts to more reliable methods such as DNA-based approaches.

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TABLES

Table 1. Number and area (m^2) of trail and traditional transects surveyed during each annual field season (2006–2008) in 3 study sites on Prince of Wales Island, Alaska.

Study Site	Trail (*n*)	Trail (m^2)	Traditional (*n*)	Traditional (m^2)	Trail overlapping[1] (n)	Trail overlapping[1] (m^2)
Maybeso	6	13,372	3	1,540	3	7,992
Staney	16	17,796	4	2,180	3	3,698
Steelhead	9	9,970	3	2,160	3	3,278

[1]Overlapping trail transects traversed the same old-growth forest patch as traditional transects.

Table 2. Pellet group densities (pellet groups/m^2 transect) along trail and traditional transects during 3 annual field seasons (2006–2008) in 3 watersheds on Prince of Wales Island, Alaska.

Study site	Year	Traditional (Pellet groups/m2 of transect)		Trail (Pellet groups/m2 of transect)		Trail overlapping[1] (Pellet groups/m2 of transect)	
		Standing	Clearance	Standing	Clearance	Standing	Clearance
Maybeso	2006	0.067	NA	0.071	0.021	0.094	0.025
	2007	0.118	NA	0.076	0.010	0.080	0.004
	2008	0.071	NA	0.060	0.021	0.078	0.030
	Mean (SE)	0.085 (0.016)	NA	0.069 (0.005)	0.017 (0.004)	0.084 (0.005)	0.013 (0.006)
Staney	2006	0.044	NA	0.078	0.021	0.089	0.018
	2007	0.085	NA	0.088	0.013	0.091	0.011
	2008	0.061	NA	0.096	0.029	0.073	0.018
	Mean (SE)	0.063 (0.012)	NA	0.088 (0.005)	0.021 (0.005)	0.084 (0.006)	0.016 (0.002)
Steelhead	2006	no data	NA	0.112	0.027	0.091	0.019
	2007	0.175	NA	0.116	0.011	0.113	0.011
	2008	0.139	NA	0.101	0.027	0.074	0.021
	Mean (SE)	0.157 (0.017)	NA	0.110 (0.004)	0.022 (0.005)	0.092 (0.011)	0.017 (0.003)
All sites	2006	0.055	NA	0.084	0.023	0.092	0.022
	2007	0.126	NA	0.091	0.012	0.090	0.007
	2008	0.094	NA	0.086	0.026	0.076	0.025
	Mean (SE)	0.095 (0.016)	NA	0.087 (0.006)	0.012 (0.001)	0.087 (0.004)	0.018 (0.006)

[1] Overlapping trail transects traversed the same old-growth forest patch as traditional transects.

Table 3. DNA-based estimates of relative deer abundance (deer/km transect) on trail transects and mark and recapture estimates of abundance (SE) reported in Brinkman *et al.* (*accepted*) during 3 annual field seasons (2006–2008) in 3 study sites on Prince of Wales Island, Alaska.

Study Site	Year	Trail (deer/km of transect)	Trail overlapping[1] (deer/km of transect)	Abundance estimates[2] (deer [SE])
Maybeso	2006	15.6	20.0	153 (10.6)
	2007	12.1	12.5	120 (9.8)
	2008	8.7	10.0	80 (7.6)
Staney	2006	14.3	20.6	180 (11.0)
	2007	12.5	8.1	157 (10.0)
	2008	11.5	6.4	137 (9.1)
Steelhead	2006	12.2	15.2	88 (7.0)
	2007	9.6	9.2	68 (6.0)
	2008	10.6	10.4	78 (6.5)
All sites	2006	14.2	19.1	421 (16.8)
	2007	11.7	10.7	345 (15.2)
	2008	10.4	9.3	295 (13.6)

[1] Overlapping trail transects traversed the same old-growth forest patch as traditional transects.
[2] Estimates of abundance were reported in Brinkman *et al.* (accepted).

Table 4. Precision of deer density estimates at varying sampling intensities and varying confidence levels was calculated using a weighted least squares regression equation derived from a boostrap resampling method. Mean pellet-group density (predictor value) was set at 0.1 PG/m^2. Precision estimates based on a study conducted on Sitka black-tailed deer in southeast Alaska during 2006–2008.

Number of transects	Area surveyed (m^2)	Confidence level	Deer density (deer/km of transect)	Precision (±deer)	Precision
12	15,924	95%	13.9	±16.0	±115%
		90%	13.9	±12.4	±89%
		85%	13.9	±10.1	±72%
25	33,176	95%	13.9	±9.8	±70%
		90%	13.9	±7.6	±55%
		85%	13.9	±6.1	±44%
50	66,352	95%	13.9	±6.3	±46%
		90%	13.9	±4.9	±36%
		85%	13.9	±4.0	±29%
75	99,525	95%	13.9	±5.3	±38%
		90%	13.9	±4.1	±30%
		85%	13.9	±3.3	±24%
93	123,411	95%	13.9	±4.5	±32%
		90%	13.9	±3.5	±25%
		85%	13.9	±2.8	±20%

FIGURES

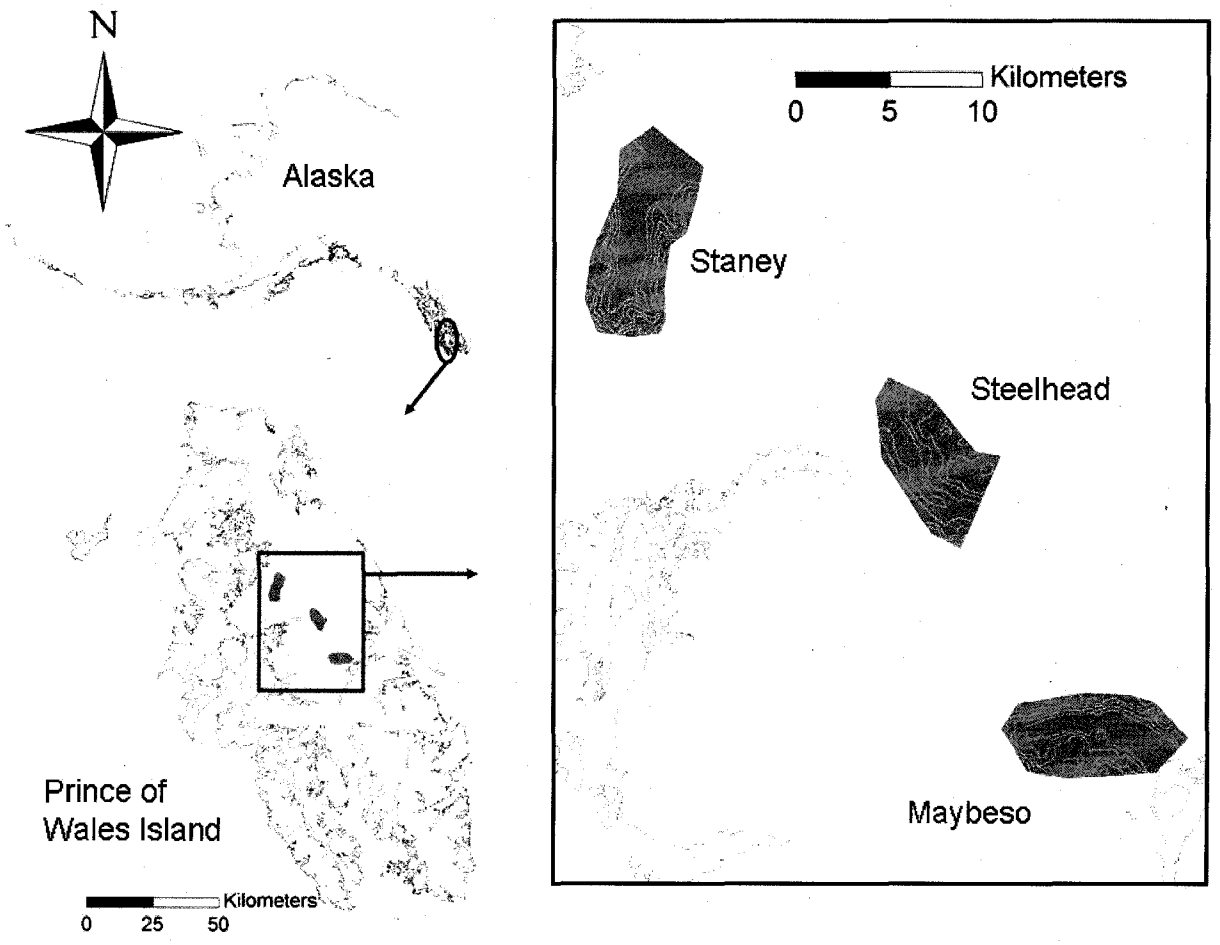

Figure 1. Location of study sites (Maybeso, Staney, Steelhead) on Prince of Wales Island in Southeast Alaska.

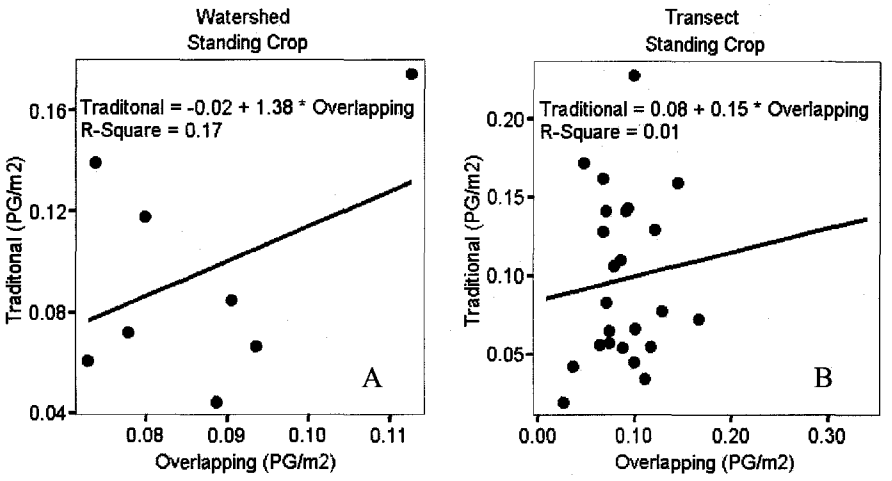

Figure 2. Watershed (A) and individual transect (B) scale relationships between standing PG densities on traditional transects and standing PG densities on overlapping trail transects (located within same forest patch as traditional transects) surveyed on Prince of Wales Island, Alaska during spring 2006–2008.

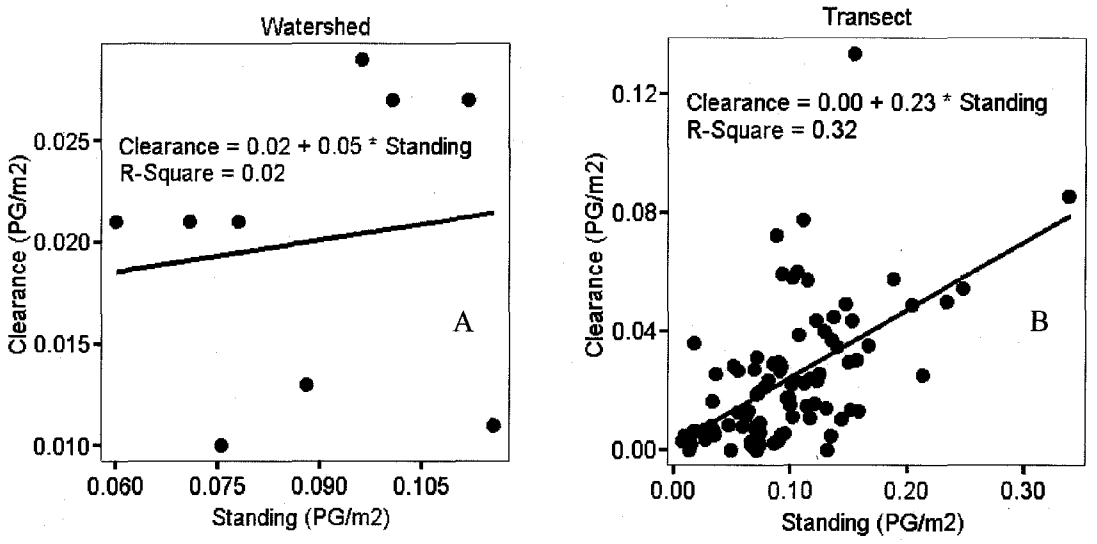

Figure 3. Watershed (A) and individual transect (B) scale relationships between standing crop PG densities (pellet groups/m^2) and clearance crop PG densities (pellet groups/m^2) deposited by Sitka black-tailed deer on trail transects surveyed on Prince of Wales Island, Alaska, during spring 2006–2008.

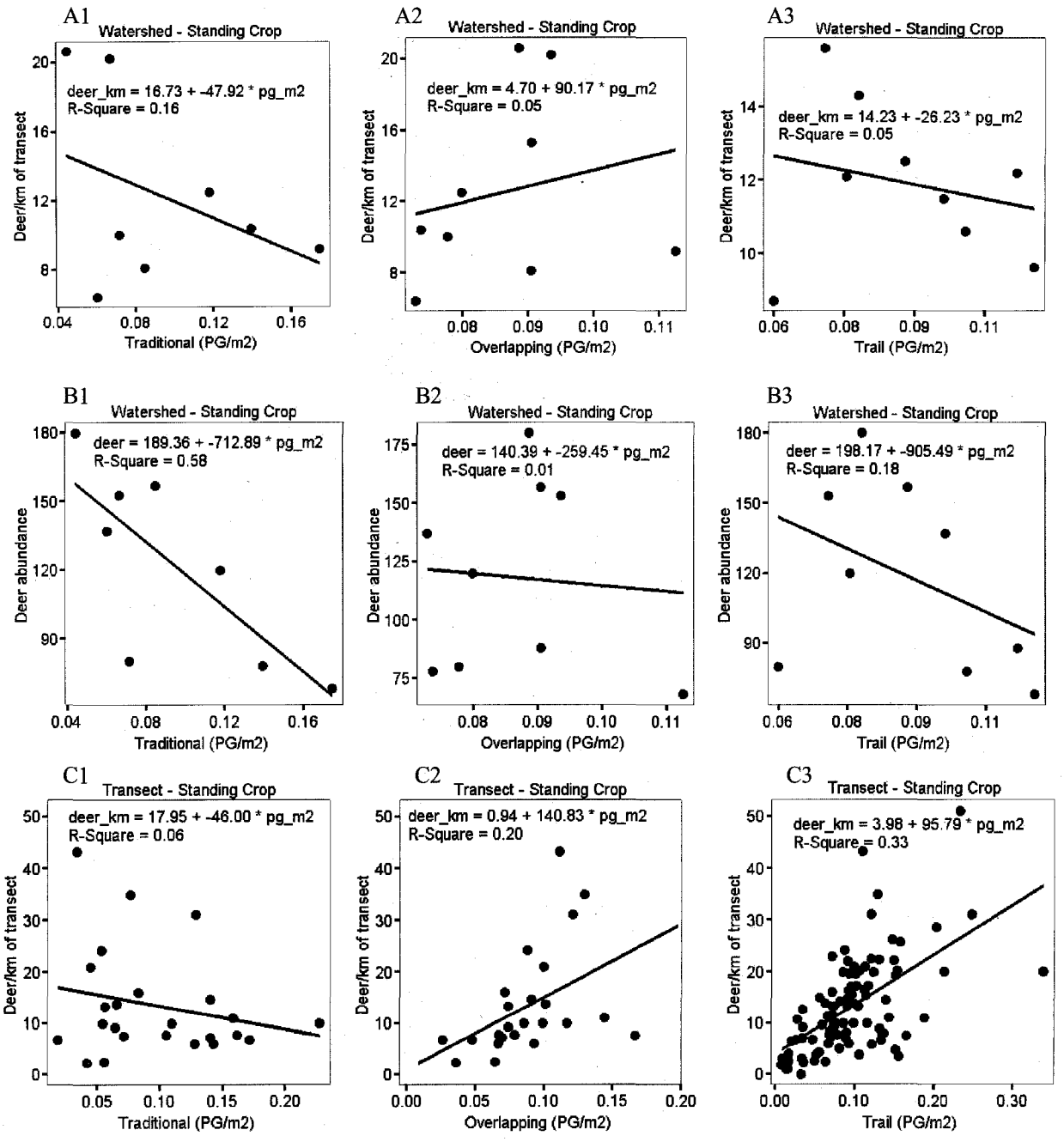

Figure 4. Watershed (A & B) and individual transect (C) scale relationships between standing crop PG densities on traditional (1), overlapping (2), and trail (3) transects, and both density (deer/km of transect) (A & C) and abundance (B) (reported in Brinkman *et al. accepted*) estimates of Sitka black-tailed deer during surveys on Prince of Wales Island, Alaska, during spring 2006, 2007, 2008.

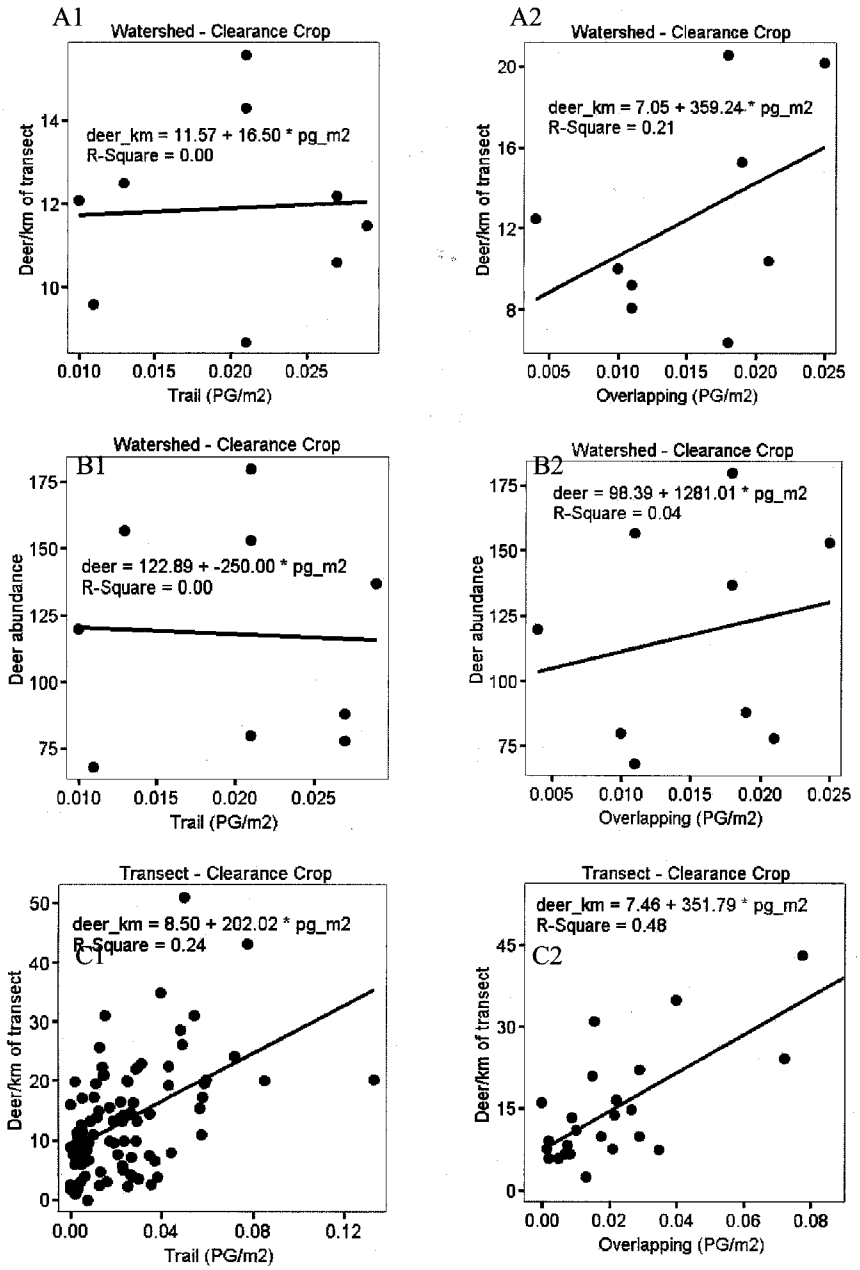

Figure 5. Watershed (A, B) and individual transect scale (C) relationships between clearance crop PG densities on trail (1) and overlapping (2) transects, and both density (deer/km of transect) (A & C) and abundance (B) (reported in Brinkman *et al. accepted*) of Sitka black-tailed deer during surveys conducted on Prince of Wales Island, Alaska, during spring 2006–2008.