

Effects of Atmospheric Pollution on High Elevation Fauna in Great Smoky Mountains National Park

Progress Report

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Theodore R. Simons and Becky Keller

USGS North Carolina Cooperative Fish and Wildlife Research Unit

Department of Zoology

Campus Box 7617

North Carolina State University

Raleigh, NC 27695

tsimons@ncsu.edu

rahylton@ncsu.edu

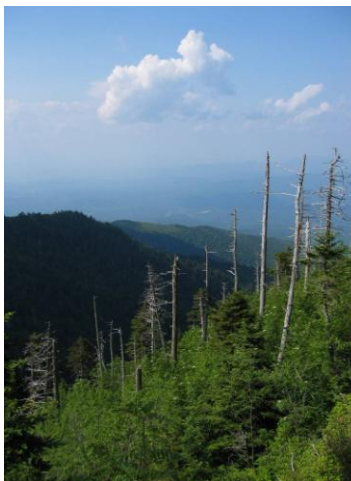


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ATMOSPHERIC POLLUTION IN THE SOUTHERN APPALACHIANS

The goal of this research is to examine the effects of atmospheric pollution on two groups of high elevation terrestrial animals. Air pollution such as acid precipitation and mercury toxicity remains high in eastern forests, and is considered a critical environmental stress (Driscoll et al. 2001, Evers et al. 2005). There may also be interactive effects between acid precipitation and mercury, as an acidified environment both enhances methylation of mercury and depletion of calcium (Harmon et al. 2003). Consequently, adverse effects may be complex (Evers et al. 2005). Thus, there is a strong need to better understand the long-term, landscape-level effects of both acid precipitation and mercury on high elevation populations.

Our research focuses on two main objectives: 1.) Quantifying the effects of acid deposition and subsequent calcium depletion on the reproductive success of high elevation songbirds and the diversity and abundance of terrestrial snail populations (the birds' primary calcium source) in the Southern Appalachians; and 2.) Determining the level of threat of mercury bioaccumulation in high elevation breeding songbirds in the Southern Appalachians. This research addresses the following hypotheses:

- H1: Terrestrial snail populations in Southern Appalachian high elevation forests have declined in areas affected by acid precipitation.
- H2: Calcium limitation associated with acid deposition negatively affects the nesting behavior and breeding success of adult Dark-eyed Juncos.
- H3: Mercury is bioaccumulating in avian populations at high elevation sites in the Southern Appalachians.

STUDY AREA

The Southern Appalachians have some of the highest annual precipitation rates in the continental United States, with annual precipitation exceeding 200 cm (NADP). The average annual acidity of rainfall in Great Smoky Mountains National Park (GRSM) is 4.5 pH, approximately 5-10 times more acidic than natural rainfall. Approximately 33% of sulfur and 50% of nitrogen deposition in the high elevations of GRSM result from clouds. Acidic clouds bathe the high elevation forests in the Southern Appalachians during much of the growing season, with an average acidity of 3.5 pH, although acidity has been measured as low as 2.0 pH. Atmospheric deposition monitoring data inside the park indicate annual wet nitrogen deposition (one of the primary elements of acid precipitation) increased 22 percent between 1981 and 1999, while calcium deposition decreased by 24 percent over the same period (National Park Service Air Resources Division 2002).

High elevation red spruce – Fraser fir habitat is located only in the Southern Appalachians, with approximately 75% located within GRSM. The red spruce-Fraser fir forest ecosystem, found only above 3500, is in jeopardy due to the synergistic effects of heavy fir loss by invasive balsam wooly adelgid beetles (*Adelges piceae*), foliar damage by high levels of ozone, and the direct and indirect effects of acid precipitation (Shortle and Smith 1988, Nodvin et al. 1995, McLaughlin and Wimmer 1999). High elevation soils are receiving such large quantities of nitrogen through acid precipitation that the ecosystem is suffering from advanced nitrogen saturation. In addition to leaching valuable nutrients such as calcium from the soil, increased nitrogen causes the release of high levels of toxic aluminum that injures vegetation and aquatic organisms such as

Brook Trout (*Salvelinus fontinalis*). Nitrate levels in some streams are approaching the public health standard for drinking water (National Park Service Air Resources Division 2002).

In 2002, year-round mercury deposition network collectors were installed in two locations in GRSM. Since then, GRSM has been identified as having some of the worst mercury contamination of nationally monitored sites, with surrounding mountainous areas expected to be similarly high. GRSM Air Resource Specialist Jim Renfro (pers. comm.) identified mercury deposition as being one of the primary threats to the area due to its high deposition rate, its tendency to bioaccumulate, and because relatively little is known about this toxin in the terrestrial system.

CALCIUM LIMITATION IN BIRDS

Introduction

Calcium is one of the cations that is stripped from the environment by acid precipitation (McLaughlin and Wimmer 1999). Reductions in environmental calcium have been tied to reductions in populations of aquatic invertebrates (Tyler and Ormerod 1992) and terrestrial snails (Graveland and van der Wal 1996), and more recently associated with reproductive problems in birds. These effects may be particularly important at high elevations (James et al. 1996), where base-poor geology limits native calcium (Shortle and Smith 1988, McLaughlin and Wimmer 1999).

Readily available environmental calcium is also extremely important for female birds during the breeding season (Dhondt and Hockachka 2001). The medullary bone, found only in female birds during reproductive seasons, offers a base of calcium storage that some birds draw from during egg laying. However, small birds have far too little

calcium available in the medullary bone to produce a clutch of eggs, and no known passerine can store more than one egg's worth of calcium in her skeleton prior to egg-laying (Graveland and van Gijzen 1994). Having very little physiological storage capacity, many passerine females collect nearly all of the calcium they need in the weeks just prior to egg laying (Graveland 1996, Taliaferro et al. 2001).

Insectivorous and granivorous birds supplement their diet with calcium-rich prey to meet their calcium needs (Graveland and van Gijzen 1994, Taliaferro et al. 2001). Calcium intake from arthropods or seeds only accounts for 5-10% of the calcium demand. A few forest passerines expand their diet to include woodlice (Isopoda) and millipedes (Diplopoda), arthropods with significantly high calcium contents (Graveland and van Gijzen 1994), but snail shells are often the main calcium rich food source found in nests of forest passerines (Graveland et al. 1994, Graveland 1996, St. Louis and Breebaart. 1993, Taliaferro et al. 2001). It is estimated that a forest passerine in New England needs eight average size snail shells (60 +/- 1.0 SE mg dry mass) in order to lay a clutch of four eggs (Taliaferro et al. 2001) or "500-600 mg of calcium to produce a clutch of nine eggs containing about 320 mg of calcium"(Graveland and Berends 1997).

Research in the Netherlands has dramatically linked the effects of acidic deposition to reductions in snail populations, thinner avian egg shells, and lower reproductive success in the Great Tit (*Parus major*), a forest passerine (Graveland et al. 1994, Graveland 1996, Graveland and Dent 1997). Nests with normal eggs contained larger snails than nests whose eggshells showed evidence of calcium deficiency (Graveland et al. 1994, Graveland 1996). Acidification has a greater effect on the abundance of calcium rich prey than it does on individual prey chemistry. Thus, food

sources high in calcium may become rare and perhaps limiting for birds breeding on acidified sites (Tyler and Ormerod 1992, Ormerod et al. 1991, St. Louis and Breebaart 1993, Graveland et al. 1994, Graveland 1996).

Female birds clearly face high calcium demands for both eggs and nestlings (Graveland et al. 1994, Graveland 1996). Females living in acidified areas without supplemented calcium greatly extend their movements and foraging effort (Ormerod et al. 1991, Tyler and Ormerod 1992). Tree Swallows traveling to calcium supplementation sites at least three km from their breeding colonies lost significantly more mass during the breeding season than birds nesting near supplementation sites (Wasson 2001). While it is possible that birds have adapted their foraging behavior and diet to meet calcium needs, the long-term costs of calcium deficiency on the fitness of adult birds have not been fully addressed.

Results to date have not always produced clear patterns. Several other studies did not find differences between calcium-rich and calcium-deficient sites when investigating the effects of calcium availability on egg condition (Ramsay and Houston 1999, Taliaferro et al. 2001), clutch size and laying date (Graveland and Dent 1997), and nestling growth (Johnson and Barclay 1996, Mahony et al. 1997, Dutta et al. 1998). In contrast, Great Tits breeding in base-poor pine forests initiated egg-laying earlier and laid larger clutches (Mand et al. 2000) than those on more acidic sites. Tilgar et al. (2005) also found that bone growth in nestling tits was positively influenced by egg calcium concentrations. The extent to which acid precipitation may be affecting avian productivity remains an open question for most species.

Surprisingly, to date only one research project has suggested that calcium limitation caused by acid precipitation may explain declines in high elevation breeding bird populations in the U.S. (Hames et al. 2001). Although the research received much attention, the results provide only correlative evidence between soil pH and declines in Wood Thrush (*Hylocichla mustelina*) populations. One significant concern with the research is that abundance estimates were based on North American Breeding Bird Surveys, a volunteer-based project in which accuracy of estimation and detection of long-term trends is considered problematic (O'Connor et al. 2000). Because the Wood Thrush project was based on little direct evidence, there is a strong need for a focused site-specific, controlled experiment to assess the mechanisms and scope of the problem of acid precipitation in high elevation forests.

Methods

We are using the Dark-eyed Junco (*Junco hyemalis carolinensis*) as a model for evaluating the effects of acid precipitation on high elevation bird communities in the Southern Appalachians. The 'Carolina Junco' is a subspecies of the Dark-eyed Junco that commonly breeds in the higher elevations in our study area. Juncos are abundant in the high elevation spruce-fir forests, and there are detailed data available on their distribution and abundance in GRSM (Shriner 2001). Juncos are primarily ground-nesters, making their nests relatively easy to locate compared to other species. They often double-brood, and calcium limitation may be greater for birds producing a second clutch. Second clutches are usually less successful than first clutches for a variety of reasons, and calcium limitation may be yet another confounding factor that reduces nesting success. Female juncos often double-brood within the breeding season, and we

would expect them to show signs of calcium deficiency before other bird species which nest only once per season (Mahony et al. 1997).

We hypothesize that calcium limitation is affecting the behavior, habitat use, and breeding success of adult Dark-eyed Juncos, and will test the following predictions: 1) calcium content of junco eggs will be negatively correlated with acid deposition; 2) calcium-supplemented birds will initiate nesting earlier than control birds; 3) calcium-supplemented birds will have larger eggs and clutch sizes, and higher hatching and nesting success than control birds.

Calcium Content in Eggs

Juncos establish and defend breeding territories from late winter through the nesting period. To test prediction 1, we collected entire clutches of 60 Dark-eyed Junco nests in high elevation areas in GRSM in spring and summer of 2006. Trails and roads throughout the spruce-fir zones served as transects for all nest searching, and nests were widely spaced (at least 200 m apart) to reduce spatial dependence among nests (Figure 1). We collected the entire clutch from all nests located. To ensure that we were collecting the entire clutch, we visited a nest multiple times before collecting if laying dates were unknown and the eggs were translucent. Junco egg shells remain translucent and fragile for a few days after incubation begins, then harden and become opaque as incubation progresses (E. Ketterson, pers. comm.).

Entire clutches were collected since laying order affects calcium content per egg within a clutch depending (J. Garlich, pers. comm.), and we were not able to record laying order as clutches were not often found before or during the egg laying process. Calcium content of egg shells was analyzed by the Department of Soil Science, NCSU in

summer 2007. We will use linear regression to analyze the relationship between average calcium content per clutch and acid deposition levels at the specific nest site.

The acid deposition data will come from a new acid deposition GIS coverage that was created by Webster et al. (in prep). Data for this model were collected by Weathers et al. (2006). Webster et al.'s spatial model improves upon Weathers et al.'s initial linear regression model by including measures of spatial dependence and prediction error (Figure 3). These models can account for patchiness of data which leads to better model fit and increased predictive power (Figure 4). Lead (Pb) deposition was used as a surrogate for acid deposition in both Weathers et al. and Webster et al.'s models, as lead deposition preserves long-term deposition patterns, including wet, dry, & cloud deposition (Weathers et al. 1995, 2000).

A core area is where birds spend approximately 95% of their time during the breeding season. Chandler et al. (1994) found the core area for Carolina Juncos to be approximately 0.38 ha (61 m x 61m). Therefore, we have chosen to estimate acid deposition using the new model by Webster et al. (in prep) at a 90 m x 90 m resolution to best represent the area surrounding the nest where birds spend most the majority of their time.

Nesting Success

To test predictions 2 and 3, we experimentally manipulated the availability of calcium to Dark-eyed Juncos over two years by providing an ad libitum calcium source (crushed oyster shell) within territories of a treatment group (Johnson and Barclay 1996, Graveland and Drent 1997, Wasson 2001, Reynolds et al. 2004). In both 2007 and 2008, we initially provided 150 g of calcium and 100 g of mixed bird seed in 30 treatment plots

and 100 g of seed only in 30 control plots. We established plot locations in 2007, alternating treatment and control plots along trails, and spacing plot centers at least 400 m apart. Initially, calcium and seed were placed only in plot centers; however, we placed additional calcium within 10 m of all junco nests located within 100 m of the plot center for the treatment group. All bird seed was sterilized by microwaving it for 3 min to avoid introducing non-native vegetation.

We actively searched for nests in treatment and control plots (15 Apr – 30 Jun 2007, 2008), visiting each plot every 3 – 5 d. We frequently found additional nests outside plot boundaries when traveling between plots. We followed the fate of all located nests, even those outside plot boundaries. We monitored timing of nest building and egg laying to determine nest initiation dates. We measured egg dimensions (length and width) and calculated mean egg size for individual clutches. We also monitored nests every 3 – 5 days to determine clutch size, hatching success, and nesting success. Nesting success was calculated using the nest survival module in Program MARK (White and Burnham 1999). Program MARK determines daily nest survival using a maximum likelihood approach that incorporates the probability of nest failure between subsequent visits. Nests were divided into three groups: those located within plots receiving calcium and seed (CALCIUM), those located within plots receiving only seed (SEED), and those that were located outside of study plots and received no supplement (OUT-OF-PLOT). We used two-tailed t-tests to examine differences in nesting success between treatment groups.

We will test for effects of supplemental calcium on clutch-initiation date, egg and clutch size, number of eggs that hatch, and number of young that fledge using Analysis of

Variance (ANOVA) or Analysis of Covariance (ANCOVA), where appropriate. We will also examine habitat variables (acid deposition, elevation, habitat type, geology) as possible covariates of nesting success using model selection procedures in Program MARK. We used 14, 12, and 26 days as the average duration of the incubation, nestling, and combined nesting stages, respectively. Average nest success per stage was determined by raising the daily nest survival rate for each period to the 14th, 12th, or 26th power, respectively.

Results

Calcium Content in Eggs

Of the junco eggs we collected, average clutch size was 3.67 ± 0.59 eggs, and eggs contained an average of 45.63 ± 3.99 (Range: 36.04 – 52.32) mg Ca per egg per nest ($n = 36$). Average Pb deposition across all nests was 43.26 ± 15.67 (range: 14.06 – 75.29) mg Pb/kg organic horizon. We found a significant decrease of 0.11 (± 0.04) mg Ca per egg per nest for every 1 mg/kg increase in Pb deposition ($F = 7.96$, $df = 1$ and 35, $P = 0.008$, $r^2 = 0.19$; Figure 2). Therefore, calcium decreased an average of 0.40 mg per nest for each 1 mg/kg increase in Pb soil deposition. Although there was some evidence of an effect of collection date on calcium content in the eggs, this was not significant.

We found no difference in clutch size between treatment ($\bar{x} = 3.84$ eggs, $N = 32$) and control groups ($\bar{x} = 3.62$, $N = 29$).

2007 Nesting Success

Across all nests monitored in 2007, an average of 35.3% of nests survived to hatching. Of those that hatched, 21.6% survived to fledge at least 1 young. Overall nesting success (likelihood of a nest producing at least 1 fledgling) across all groups in

2007 was 7.6% (Table 1). Overall, daily nest survival was higher during the incubation period compared to the nestling period (t-test, $P = 0.020$; Table 1). This higher mortality during the nestling period was likely due to the increased activity of adults feeding young and/or begging calls by the chicks. Nest failures appeared to result from numerous causes; however depredation was the leading factor. Fifty nests (69%) were depredated, and nests were often found pulled out of their holes and/or destroyed. In one instance, an entire root ball where a nest was located was destroyed, suggesting a bear as the predator. We observed a snake in another depredated nest. In two other instances, plucked adult flight feathers were found scattered around the nests, indicating adult mortality by an avian predator. Six nests (8%) were abandoned during incubation due to unknown causes.

Our preliminary results indicate that nest survival in 2007 was not significantly different between CONTROL, SEED, and OUT-OF-PLOT groups (Tables 1 and 2). Daily survival during the incubation stage was not significantly different between CALCIUM and SEED plots ($P = 0.914$), CALCIUM and OUT-OF-PLOT ($P = 0.361$), nor SEED and OUT-OF-PLOT ($P = 0.308$). Daily survival during the nestling stage was not significantly different between CALCIUM and SEED plots ($P = 0.770$), CALCIUM and OUT-OF-PLOT ($P = 0.730$), nor SEED and OUT-OF-PLOT ($P = 0.386$). Daily survival during the overall nesting stages (incubation and nestling stages combined) was not significantly different between CALCIUM and SEED plots ($P = 0.942$), CALCIUM and OUT-OF-PLOT ($P = 0.279$), nor SEED and OUT-OF-PLOT ($P = 0.227$).

2008 Nesting Success

Across all nests monitored in 2008, an average of 41.3% of nests survived to hatching. Of those that hatched, 47.3% survived to fledge at least 1 young. Overall nesting success (likelihood of a nest producing at least 1 fledgling) across all groups in 2007 was 25.5% (Table 3). Unlike in 2007, daily nest survival in 2008 was not higher during the incubation period compared to the nestling period (t-test, $P = 0.73$; Table 1). A total of 21 nests (40%) were depredated, and 10 nests (19%) were abandoned due to unknown causes.

As seen in 2007, preliminary results indicate that nest survival in 2008 was not significantly different between CONTROL, SEED, and OUT-OF-PLOT groups (Tables 3 and 4). Daily survival during the incubation stage was not significantly different between CALCIUM and SEED plots ($P = 0.997$), CALCIUM and OUT-OF-PLOT ($P = 0.985$), nor SEED and OUT-OF-PLOT ($P = 0.925$). Daily survival during the nestling stage was not significantly different between CALCIUM and SEED plots ($P = 0.777$), CALCIUM and OUT-OF-PLOT, ($P = 0.504$) nor SEED and OUT-OF-PLOT ($P = 0.677$). Daily survival during the overall nesting stages (incubation and nestling stages combined) was not significantly different between CALCIUM and SEED plots ($P = 0.962$), CALCIUM and OUT-OF-PLOT ($P = 1.000$), nor SEED and OUT-OF-PLOT ($P = 0.962$).

Additional results will be forthcoming after examining the effects of environmental covariates on nesting success over both years.

Discussion

Effects of acid deposition and subsequent calcium limitation on birds have proved inconsistent in Europe and North America, and this pattern holds for our study. We

detected a significant decrease in calcium content in junco eggs as deposition increased, however clutch size and nesting success do not seem to be influenced by current levels of acid deposition in GRSM.

The overall nesting success for juncos in this study varied between 10.5% in 2007 and 25.5% in 2008. Although these values seem low, they are similar to those found in other studies of *Junco hyemalis carolinensis*. Like many ground-nesting birds, juncos are known to have low nesting success (Ketterson et al. 1996), which appears to be largely a function of small-mammal population fluctuations. Our observed nest predation rates of 69% (2007) and 40% (2008) fall within the range (20-85%) observed at Mountain Lake Biological Station (Nolan et al. 2002). Our nest visits may have negatively affected nesting success; however we attempted to reduce our impact by not leaving direct scent trails to nests, and using sticks to lift any vegetation obscuring nest contents. We also made an effort to not linger by nests, to touch vegetation away from nests, and to be aware of possible predators watching our movements. Thus, these data appear sufficient for testing predictions related to calcium limitation.

We did not detect significant differences in daily nesting survival between any of the treatment groups during any nesting stage in either year. These results are preliminary however, and we have not tested the effects of adding environmental covariates to the model, which may significantly reduce the large variability observed, especially within the CALCIUM group. Sample sizes within a given year were often small as well, which may have precluded detecting small differences in daily nest survival.

CALCIUM LIMITATION IN SNAILS

Introduction

Research in the Netherlands has dramatically linked the effects of acidic atmospheric deposition to reductions in snail populations, and subsequent reductions in bird populations, as snail shells are the primary source of calcium for many songbirds (Graveland et al. 1994, Graveland 1996, Graveland and Dent 1997). Calcium demands are especially high in terrestrial snails during shell formation (the shell is primarily composed of calcium carbonate) and egg laying (Fournie and Chetail 1984). Snail abundance and species richness are often limited by low soil pH and low calcium content in the soil or litter (Wareborn 1992, Hotopp 2002, Millar and Waite 2002, Martin and Sommer 2004). Research in Europe and North America has shown that recent changes in soil chemistry due to increased atmospheric acid deposition have significantly reduced total snail abundance and species richness (Wareborn 1992, Graveland et al 1994, Graveland and van Gijzen 1994, Hotopp 2002). Artificial liming of acidic soils has also been shown to increase snail abundance (Graveland and van der Wal 1996, Johannessen and Solhoy 2001).

The Southern Appalachians host the greatest diversity of land snails in the United States. Approximately 13 terrestrial snail species inhabit the high elevation red spruce-Fraser fir forests in Great Smoky Mountains National Park, Blue Ridge Parkway, and the surrounding National Forest lands, many of which are endemics to Southern Appalachian spruce-fir forests. Laboratory studies indicate that calcium availability significantly affects reproduction and growth of terrestrial snails (Gomot et al. 1989, Ireland 1991, Wacker and Baur 2004). Snail density and species richness have also been significantly

linked with calcium content of litter and soil (Wareborn 1970, Gardenfors 1992, Graveland and van der Wal 1996).

Anecdotal evidence (J. Slapcinsky, pers. comm.) indicates that snail populations in the study area are dramatically declining, while land slug populations may not be. Slugs are closely related to snails and inhabit similar ecological niches, yet their calcium needs are greatly reduced as they do not excrete a calcareous protective shell. Although the lack of historic abundance estimates of snails or slugs limits true comparisons with current populations, comparing the relative abundance of slugs to snails across the area would isolate the question of calcium limitation for snails.

This research will address the following hypothesis: terrestrial snail populations in high elevation forests in the Southern Appalachians are negatively affected by acid precipitation. We predict that 1) terrestrial snail abundance and species richness are limited by soil pH, acid deposition, and calcium availability; and 2) terrestrial snail shell size, thickness, and calcium content have decreased over the past century.

Methods

Species composition

Terrestrial snail species located in the Fraser fir/red spruce high elevation forests in the Southern Appalachians include *Fumonelix wheatleyi* (Clingman Covert), *Haplotrema kendeighi* (Blue-foot Lancetooth), *Inflectarius ferrissi* (Smoky Mountain Covert), *Mesodon altivagus* (Wandering Globe), *Mesodon andrewsae* (Balsam Globe), *Mesodon normalis* (Grand Globe), *Mesomphix andrewsae* (Mountain Button), *Mesomphix subplanus* (Flat Button), *Stenotrema depilatum* (Great Smoky Slitmouth), *Stenotrema altispira* (Highland Slitmouth), *Ventridens acerra* (Golden Globose Dome),

Ventridens arcellus (Golden Dome) and *Vitrinizonites latissimus* (Glassy Grapeskin).

We also infrequently collected two species in lower elevation spruce-dominated sites:

Paravitrea clappi (Mirey Ridge Supercoil) and *Glyphyalinia sculptilis* (Suborb Glyph).

Snails were identified based on the mollusk identification guides of Pilsbry (1939, 1940, 1946, 1948), Burch (1962) and Dourson and Dourson (2006).

Field Study

We used timed searches within red spruce-Fraser fir forests in Great Smoky Mountains National Park (GRSM) in 2006 and 2007 to collect current samples of high elevation terrestrial gastropods. To test prediction 1, we used these samples to test for differences in abundance or species richness among sites across an acid deposition gradient. To test prediction 2, we will use morphometric measurements of these samples to compare to historic measurements using museum specimens (see Museum Study below).

We used the GRSM acid deposition model created by Weathers et al. (2006; Figure 4) to choose study sites in the National Park. We collaborated with M. Jenkins (Plant Ecologist, GRSM) and S. Stehn (Denali National Park) in choosing sites, as Jenkins and Stehn conducted a vegetation study from 2007-2008 in the same areas to look at the effects of acid precipitation on plant communities in the park. Stehn's crew visited each site one to two years after we conducted the timed searches, and has recently shared vegetation and soil data with us.

We intensively sampled snails within GRSM at 60 sites randomly selected within a predefined habitat and acid deposition gradient stratification scheme. Because estimated sulfur deposition ranges between 10-42 kg/ha/yr in the spruce-fir zone

(Weathers et al. 2006), we chose 15 sites within each of 4 deposition bands (Figure 2). Sites were further stratified into habitat classifications, with 10 fir and 5 spruce sites within each deposition band. Fir habitat was defined as any site that included Fraser fir as a dominant species in the overstory vegetation category using the 2004 NatureServe vegetation model for GRSM (White et al. 2003). Using the same classification model, spruce habitat was considered all areas that included Red Spruce as a dominant species in the overstory vegetation category which did not also include Fraser Fir as a dominant species. Sites that had both Fraser fir and red spruce as dominant overstory species were placed in the fir category as Fraser fir has a more restricted range (Figure 2). Site locations that were inaccessible due to steep terrain and related safety issues were discarded and new sites were randomly selected within the same habitat-acid deposition stratum. Sites were restricted to being at least 200 m apart to reduce spatial dependence.

We collected samples from 33 of the 60 sites during June – July 2006, including at least 2 sites from each habitat-acid deposition stratum. The remaining sites were sampled from June – July 2007. We established a 400 m² sample plot that was centered at each of the predetermined site's coordinates. A few plots were slightly shifted (2-10 m) to ensure that all plot boundaries were at least 10 m from trails, roads, or obvious edges (e.g. clearing). Plot boundaries were flagged and marked with painted rebar, and two witness trees were flagged and recorded for the upper left corner of each plot. These plots were reused by GRSM personnel in Stehn's related vegetation survey in 2007 and 2008.

We conducted 40-minute 2-person (1.3 search hours) timed searches of all leaf litter, rocks, woody debris, and live plant stems within each plot (Graveland and van der

Wal 1996). This amount of time allowed for a thorough search of the entire plot, which we felt was necessary due to potential patchiness of snail distributions within plots as well as terrain variability. All slugs and snails within a plot were collected and stored together in a polyurethane jar containing museum-grade 95% nondenatured ethanol (Sturm et al. 2006). The ethanol was reapplied after 24-48 h to prevent a dilution effect.

We are currently analyzing collected snail and slug species from the field study to species level, as alive or dead, and adult or juvenile when possible. Many of the snail species we are working with develop a calcareous lip when fully grown, and we are using this characteristic to define age of the snails when possible. We are measuring maximum height and length of each shell, as well as number of whorls. We will use multiple regression to examine the relationship between snail density or species richness and soil pH, soil calcium content, vegetation characteristics, and acid deposition.

Museum Study

For prediction 2, we will compare current shell morphometrics of adult terrestrial snails from our study sites with those of adult museum specimens. We are collecting morphometric data on each collected snail, including number of whorls, maximum height, maximum width, and average shell thickness (approximately 5 mm inside the aperture, measurement repeated 3 times) using electronic calipers (Mitutoyo Model 500-682) with 0.02 mm accuracy. All snails will be analyzed for calcium content at North Carolina State University in the Department of Soil Sciences after dissolving in a strong acid. Understanding total calcium content in relation to shell morphometrics will allow us to estimate calcium content of museum specimens (see below) without using destructive techniques.

Preliminary research reveals that museums will provide a rich source of data on the terrestrial snail species inhabiting the high elevation forests of GRSM. Although on-line databases of museum collections are rare, the Field Museum of Natural History, Chicago, and the Florida Field Museum of Natural History, Gainesville, both have detailed information on all gastropods in their collection. From these databases alone, we determined that there are sufficient samples (> 20) from each of 3 time periods (early: 1930-1940; middle: 1950-1970; late: 1980 - present) to develop a history of changes in snail shell quality in at least five species of snails, and to make comparisons with current snail shells collected in GRSM. There are additional museums with extensive snail collections, and we anticipate a thorough on-site investigation would increase both sample size and number of species that could be evaluated for changes in shell quality.

Results and Discussion

We have begun measurements of snails from both the field and museum studies; however this is a lengthy process that will continue throughout 2010. We have currently taken measurements of *Mesodon altivagus* ($n = 56$), *Mesodon andrewsae* ($n = 54$), and *Inflectarius ferrissi* ($n = 30$) museum specimens from the Florida Museum of Natural History. Preliminary results are being analyzed to determine additional number of specimens that we will need to borrow from other museums.

MERCURY BIOACCUMULATION IN BIRDS

Introduction

Mercury (Hg) and methylmercury (MeHg) are important contaminants of wildlife populations (Burger 1993, Wolfe et al. 1996, Nichols and Bradbury 1999, Evers et al. 2005). Inorganic mercury enters aquatic systems via atmospheric deposition (Mierle

1990, Wier et al. 1990), where it is chemically converted to MeHg, a biologically active and toxic form. Research has shown that MeHg bioaccumulates through the aquatic foodweb from plankton, to invertebrates, to fish, and then to piscivorous vertebrates (Frederick et al. 1999, Evers et al. 2005). Although recently there has been much improvement in understanding the mechanisms of mercury transfer and accumulation in freshwater aquatic systems (Evers and Clair 2005, US EPA 2002), little is known about its role in the terrestrial system.

Upland forest soils are natural sinks for atmospherically deposited mercury because the majority of mercury binds to organic and mineral soil particles (Hall and St. Louis 2004). Mercury is concentrated primarily in the upper soil column (Kim et al. 1997). Research indicates that up to 60% of Hg that reaches lakes originates from the terrestrial watershed (Krabbenhoft and Babiarz 1992, Griegal 2002). Mercury loading is significantly higher in evergreen compared to deciduous habitats, and higher (2-5x) in mountainous areas in the northeastern United States compared to nearby low elevation areas (Lawson 1999; Miller et al. 2005). Consumption of contaminated food is the primary risk of mercury exposure to terrestrial vertebrates.

Birds are at particularly high risk to mercury toxicity because many species are at high trophic levels, are long-lived, and are vulnerable to neurological and reproductive impacts from elevated Hg levels (Evers et al. 2005). The effects of mercury contamination in birds has been primarily studied in large migratory birds, wading birds, shorebirds, and raptors; few studies have addressed these issues in passerines (Bishop et al. 1995, Rosten et al. 1998, Reynolds et al. 2001), especially those species not associated with aquatic systems. In a multi-species analysis of mercury contamination, piscivorous

birds were repeatedly shown to have some of the highest MeHg levels of the 38 species analyzed, however insectivorous birds in both aquatic and terrestrial habitats were also found with elevated levels (Evers et al 2005).

Although little research has focused on effects of mercury in passerines, Rimmer et al. (2005) hypothesized that among terrestrial birds mercury contamination poses the highest risk to obligate insectivorous species. Rimmer et al. (2005) found that blood mercury levels in Bicknell's Thrush (*Catharus bicknelli*) were correlated with Hg levels in leaf litter, demonstrating that dietary MeHg uptake occurs in passerines inhabiting terrestrial environments unassociated with standing water. Mercury accumulation in nestling Prothonotary Warblers (*Protonotaria citrea*) was also correlated with soil mercury concentrations around the nest (Adair et al. 2003). Rimmer et al. (2005) further demonstrated that blood Hg levels in Bicknell's Thrush overlapped with those of Bald Eagles, indicating equivalent trophic status of a terrestrial-based insectivore with an aquatic-based piscivore.

Rosten et al. (1998) found that resident insectivorous passerine species at contaminated sites in Europe accumulated higher mercury loads in eggs and nestlings than migratory or seasonal species. Although Bishop et al. (1995) claims that nestling birds are the best indicator of local contaminant trends, mercury levels in adult resident birds can also demonstrate chronic effects due to bioaccumulation. One limitation with the Rimmer et al. (2005) study is that they examined mercury levels in Neotropical migrants, and were thus unable to study chronic effects of mercury in resident adults.

Because an acidified environment both enhances methylation of mercury (Harmon et al. 2003) and depletion of calcium, the adverse effects of acid precipitation

may be complex. A recent study showed that Pied Flycatchers (*Ficedula hypoleuca*) were more sensitive to decreased levels of calcium-rich prey (i.e. snails) in a heavy metal polluted area (Eeva et al. 2005). Thus, there is a strong need to better understand the long-term, landscape-level effects of atmospheric pollution on high elevation bird populations (Evers et al. 2005).

The primary objectives of this research are to examine the threat of mercury bioaccumulation in high elevation songbirds in Great Smoky Mountains National Park (GRSM), and to test for the relationship between mercury bioaccumulation and trophic position in these birds. GRSM experiences some of the worst atmospheric mercury contamination of nationally monitored sites. Mercury is a main threat to the area due to its high deposition rate, its tendency to bioaccumulate, and because relatively little is known about this toxin in the terrestrial system (J. Renfro, pers. comm.).

We hypothesized (Hypothesis 1) that mercury is bioaccumulating in avian populations at high elevation sites in the Southern Appalachians. We predict that 1) feather mercury levels of year-round resident adult songbirds will be higher in areas of increased deposition; 2) feather mercury levels of nestling songbirds will be higher in areas of increased deposition; 3) blood mercury levels of adult high elevation passerines will be higher in areas of increased deposition; 4) mercury levels in birds in high elevation forests are associated with habitat and topographic features; and 5) feather mercury levels of birds in the Southern Appalachians will exceed concentrations found to cause adverse effects in avian species.

We also hypothesized (Hypothesis 2) that mercury levels in birds are tied to their trophic position, such that birds with higher stable nitrogen isotope ratios ($\delta^{15}\text{N}$) would

exhibit higher mercury levels. Stable isotopes can be used to evaluate an animal's relative trophic position in a food web by determining diet composition (Pearson et al. 2003), as the nitrogen and carbon isotopic signatures of prey items influence the isotopic signatures of a consumer's tissues (Vander Zanden and Rasmussen 1999). Further, trophic position based on isotopic signatures has been successfully used to predict mercury concentrations in seabirds (Atwell et al. 1998).

Overall, this research will significantly strengthen GRSM's ability to develop a comprehensive research plan for mercury, determine the mechanism of exposure to wildlife, and will be invaluable for examining the threat of mercury to our wildlife and determining habitats and species that are most at risk.

Methods

In birds, mercury loads are often sampled in specific organs, blood, or feathers, with mercury accumulation seen regardless of the material examined (Burger 1993). Although adult females can reduce body burdens of mercury during egg production, depuration of MeHg most commonly occurs during feather growth, as MeHg binds strongly to keratin (Crowther et al. 1965). Virtually all Hg in a feather is MeHg (Thompson et al. 1989, Frederick et al. 2004) and reflects blood Hg levels at the time of molt (Bearhop et al. 2000). We sampled both blood and feathers for this study. As MeHg typically represents 90-100% of total Hg loads in birds, we are analyzing feathers and blood for total Hg (Burger 1993). Because the feathers of neotropical migrants often reflect Hg burdens from the nonbreeding grounds where they last molted their feathers, we will analyze blood instead of feathers for Hg in adult migrant species to test

prediction C, as blood Hg values represents short-term Hg uptake from the local environment (Evers et al. 2005).

Mercury Bioaccumulation and Environmental Variables

In an effort to select banding sites across a wide deposition gradient, we chose sites using the GRSM acid deposition model (Weathers et al. 2006) as a surrogate for mercury deposition, as deposition mechanisms for mercury are likely similar to those for sulfur, nitrogen, and lead (K. Weathers, pers. comm.). We collected samples for mercury testing from a total of 44 bird species ($n = 398$ birds) across 34 sites in GRSM and along the Blue Ridge Parkway during 2006 – 2008 using passive mist netting (Table 5). We will use the improved deposition model by Webster et al. (in prep) to calculate deposition rate, which will be used as a covariate in the analysis of covariance (ANCOVA) to compare Hg loads across banding sites for predictions 1, 2, and 3 of Hypothesis 1.

Dark-eyed Juncos are the most common species captured at high elevation sites and accounted for approximately half our captures. Thus, we concentrated on increasing our sample size for juncos in 2008, and collected 129 additional junco feather samples for mercury analysis at 19 new locations which will be added to existing data to better understand the spatial differences in mercury bioaccumulation (prediction 4).

Each bird was banded with an individually numbered USFWS band, and the following measurements were recorded: age, sex, mass, wing cord, flight feather and body molt, and brood patch, cloacal protuberance, and fat scores. We collected feather samples by pulling both S2 secondary flight feathers, which were stored in clean, dry paper envelopes. Blood was collected from the brachial vein using heparinized capillary tubes and stored in a cooler until returning to our field house. Blood and feather samples

were collected only from healthy individuals. Blood samples were collected primarily from adults, while feather samples were collected from both adults and juveniles. Blood and feathers were collected from juveniles only after they were fully flighted (i.e. no flight feathers in sheath).

One of the two secondary feathers will be analyzed at the North Carolina State University Department of Environmental and Molecular Toxicology using cold vapor atomic fluorescence spectroscopy (EPA method 1631). Blood samples will be analyzed by cold vapor absorption spectroscopy (EPA method 245.7) at Texas A&M Trace Elements Lab. To test predictions 1 – 4, we will use Analysis of Covariance (ANCOVA) to compare Hg loads, with deposition, habitat, or topographic features as a covariate, where appropriate. To test prediction 5, we will compare feather total mercury values to standard concentrations known to cause neurological and reproductive problems in birds.

Mercury Bioaccumulation and Trophic Status

At each of the 34 banding sites, we collected three composite samples (at least three individuals) of two guilds of invertebrates (Lepidoptera and Diptera) to serve as baseline $\delta^{15}\text{N}$ values for comparison to the isotopic signatures in the birds' tissues (Vander Zanden 1996). We sampled invertebrates by beating tree branches and other vegetation over a 1 x 1 m white sheet for three minutes at three locations within each site. These invertebrate guilds were selected to achieve baseline isotope signatures as they serve as common prey items for nestling birds.

We will use the remaining S2 secondary feathers collected in 2007 and 2008 ($n = 233$) for stable isotope analysis. These feathers will be analyzed for stable isotopes $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ at North Carolina State University using a 20-20 mass spectrometer following

the methods described in Post et al. (2000). We will test Hypothesis 2 using Analysis of Variance (ANOVA) to compare feather Hg loads to trophic status.

Results

We are waiting for additional funding to analyze feather and blood samples from 2008. Feathers collected in 2006 had an average total mercury load of 476 $\mu\text{g/kg}$ (range 18 - 1,548 $\mu\text{g/kg}$, $n=219$). Feathers collected in 2007 had an average total mercury load of 393 $\mu\text{g/kg}$ (range 5 – 3,744 $\mu\text{g/kg}$, $n=135$). Feather total mercury loads (mean $\pm$ SE) in 2006 were significantly higher in juvenile (504 ± 25 $\mu\text{g/kg}$, $n=150$) versus adult (415 ± 29 $\mu\text{g/kg}$, $n=69$) birds when all species were combined ($p=0.02$). Feather mercury loads in juvenile Dark-eyed Junco (557 ± 44 $\mu\text{g/kg}$, $n=53$) were significantly higher in 2006 compared to adults (351 ± 28 $\mu\text{g/kg}$, $n=50$, $p<0.0001$). Feather mercury levels in juvenile of 10 high elevation resident species (528 ± 32 $\mu\text{g/kg}$, $n=99$) were not significantly higher than those in juveniles of 11 high elevation migrant species (455 ± 17 $\mu\text{g/kg}$, $n=43$, $p=0.18$) in 2006. We will continue analyzing these data in 2009, in particular investigating the patchiness of high mercury values such as seen at Indian Gap and Oconoluftee Overlook in 2006 (Table 3).

In 2006, species with the highest feather mercury loads (>1000 $\mu\text{g/kg}$) included Winter Wrens (*Troglodytes troglodytes*), Veery (*Catharus fuscescens*), Dark-eyed Junco, Canada Warbler (*Wilsonia canadensis*), Golden-crowned Kinglet (*Regulus satrapa*), Song Sparrow (*Melospiza melodia*), Black-throated Blue Warbler (*Dendroica caerulescens*), and Brown Creeper (*Certhia americana*). The Indian Gap site consistently had the highest mercury levels in juvenile feathers in 2006 (Table 3).

Species with the highest feather mercury loads ($>1000 \mu\text{g/kg}$) in 2007 included Carolina Wren (*Thryothorus ludovicianus*), Golden-crowned Kinglet, Ruffed Grouse (*Bonasa umbellus*), Indigo Bunting (*Passerina cyanea*), Eastern Towhee (*Pipilo erythrophthalmus*), and Dark-eyed Junco. The feather mercury level in the Carolina Wren was more than double the highest amount seen in any other individual in GRSM to date at $3744 \mu\text{g/kg}$ (Table 4).

We sampled many fewer species in 2008 than in prior years, instead focusing on sampling Dark-eyed Juncos across a wide area. However, species with some individuals showing mercury loads $>1000 \mu\text{g/kg}$ in 2008 included Carolina Wren, Winter Wren, Slate-colored Junco, Golden-crowned Kinglet, and Sharp-shinned Hawk (*Accipiter striatus*). A Golden-crowned Kinglet had the highest level in 2008, at $2,052 \mu\text{g/kg}$ total mercury.

Further results from this study are pending statistical analysis in 2010, as well as feather and caterpillar stable isotope analysis.

Discussion

Our results provide the first direct evidence of mercury contamination to GRSM wildlife. Our results indicate that high elevation terrestrial songbirds in the Southern Appalachians are accumulating mercury as originally seen in the northeastern U.S. (Rimmer et al. 2005), which mercury experts would previously not have predicted given the low percentage of wetlands in the mountainous area. Feather total mercury loads were significantly higher in juvenile versus adult birds, indicating contamination at a critical period in the birds' lives.

The highest mercury levels ($> 1\text{ppm}$) were typically found in high elevation invertivores. Birds in the lower elevations averaged much lower mercury levels compared to the high elevations except for Carolina Wrens (*Thryothorus ludovicianus*). One Carolina Wren captured at Fontana Lake in 2007 had mercury levels exceeding the accepted neurological and behavioral problem threshold of 3 ppm (Evers et al. 2005). The high mercury levels in the Carolina Wren is very interesting, as this species is known to incorporate higher proportions of spiders in its diet than other species, which might indicate their relative higher trophic position (D. Evers, pers. comm.). The capture location of the Carolina Wren by the lake also suggests that the source of mercury could be partially bioaccumulating through the aquatic food web, such as by eating prey items which spend their larval stage in the aquatic system before emerging as adults. Other lower-elevation species associated with water also demonstrated some of the highest mercury loads: Belted Kingfisher, Louisiana Waterthrush, and Yellow-breasted Chat.

We will conduct further statistics to examine environmental covariates which may help explain the spatial patchiness observed in our data. Dark-eyed Juncos will serve as an excellent model for this analysis as they are year-round residents, are wide-spread in the high elevations, and account for approximately half of our captures.

Mercury has recently been identified as a serious concern in GRSM; however, relatively little is known about this pollutant. Studying mercury in terrestrial birds is an emerging field, and this research will lay the foundation for additional studies, both in birds and other wildlife species. The stable isotope analysis will be used to link mercury accumulation in birds to food web structure, a critical link currently lacking in studies of mercury in the terrestrial system. Understanding which watersheds and/or habitats are

most likely to see bioaccumulation of mercury will significantly strengthen our knowledge of mechanisms involved in mercury contamination in the Southern Appalachians. In addition, understanding the role of Hg in terrestrial systems could have world-wide implications as data on this topic are extremely limited, and could provide the basis for establishing new environmental thresholds.

SIGNIFICANCE OF RESEARCH

This research project combines both exploratory and experimental components and will provide direct means of assessing the effects of acid deposition, Ca limitation, and Hg pollution on songbird and land snail populations in Southern Appalachian high elevation forests. We believe this project has a high probability of success because many of the research questions and methods have been well defined through previous studies in Europe, and because there is strong support for the work from the National Park Service and our many collaborators. The large number of collaborations we have developed speaks to the diverse interest in this project across multiple disciplines, as well as our commitment to its success. Overall, these collaborations have connected a diverse array of organizations and agencies, and have significantly increased interest in pollution-related research in the Southern Appalachians.

This research will have a broad impact on the field of applied ecology, offering direct recommendations for environmental conservation and management. We feel that it is important to disseminate our results to the public as well as resource managers, and songbirds have proven to be excellent model species for conveying the significance of research findings to the public. Results from this project will be applicable across the southeast as well as the entire Appalachian Mountain Range. In addition, understanding

the role of Hg in terrestrial systems could have world-wide implications as data on this topic are extremely limited, and could provide the basis establishing new environmental thresholds.

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Table 1. Dark-eyed Junco overall nest survival across groups in 2007.

Nesting Stage	Calcium & Seed	Seed	Out-of-Plot	Combined Groups
Incubation	0.405	0.459	0.187	0.353
Nestling	0.279	0.217	0.124	0.216
Entire season	0.135	0.154	0.036	0.105

Table 2. Dark-eyed Junco daily nest survival rates ($\pm$ SE) across groups in 2007.

Nesting Stage	Calcium & Seed	Seed	Out-of-Plot	Combined Groups
Incubation	0.945 $\pm$ 0.016 ($n = 23$)	0.953 $\pm$ 0.015 ($n = 21$)	0.900 $\pm$ 0.027 ($n = 19$)	0.937 $\pm$ 0.011 ($n = 63$)
Nestling	0.899 $\pm$ 0.036 ($n = 11$)	0.887 $\pm$ 0.034 ($n = 19$)	0.840 $\pm$ 0.053 ($n = 10$)	0.880 $\pm$ 0.023 ($n = 40$)
Entire season	0.931 $\pm$ 0.015 ($n = 22$)	0.935 $\pm$ 0.014 ($n = 28$)	0.888 $\pm$ 0.024 ($n = 22$)	0.923 $\pm$ 0.010 ($n = 72$)

Table 3. Dark-eyed Junco overall nest survival across groups in 2008.

Nesting Stage	Calcium & Seed	Seed	Out-of-Plot	Combined Groups
Incubation	0.424	0.420	0.394	0.413
Nestling	0.564	0.486	0.383	0.473
Entire season	0.249	0.264	0.254	0.255

Table 4. Dark-eyed Junco daily nest survival rates ($\pm$ SE) across groups in 2008.

Nesting Stage	Calcium & Seed	Seed	Out-of-Plot	Combined Groups
Incubation	0.948 $\pm$ 0.192 ($n = 18$)	0.947 $\pm$ 0.023 ($n = 11$)	0.944 $\pm$ 0.021 ($n = 16$)	0.946 $\pm$ 0.012 ($n = 45$)
Nestling	0.953 $\pm$ 0.026 ($n = 10$)	0.942 $\pm$ 0.028 ($n = 10$)	0.923 $\pm$ 0.033 ($n = 13$)	0.939 $\pm$ 0.017 ($n = 33$)
Entire season	0.952 $\pm$ 0.014 ($n = 19$)	0.953 $\pm$ 0.015 ($n = 16$)	0.952 $\pm$ 0.014 ($n = 23$)	0.952 $\pm$ 0.008 ($n = 58$)

Table 5. Songbird netting location information for feather and blood collection within Great Smoky Mountains National Park (GRSM) and Blue Ridge Parkway (BLRI) from 2006-2008.

Site	Year	Site Description	Latitude	Longitude
BKRT	2007	Black Rock Trailhead, BLRI	35.7153	82.2742
CACO	2007	Cades Cove, GRSM	35.5978	83.8258
CLDO	2006	Clingman Dome, GRSM	35.5642	83.4986
COGA	2007	Coopers Gap, GRSM	35.5775	83.4803
EACR	2007	Eagle Creek Campground, GRSM	35.4844	83.7744
FORK	2007, 2008	Fork Ridge Trailhead, GRSM	35.5919	83.4694
GSMI	2006, 2007, 2008	Great Smoky Mountains Institute at Tremont, GRSM	35.4772	83.6894
HACR	2008	Hazel Creek Campground, GRSM	35.4736	83.7267
INGA	2006	Indian Gap, GRSM	35.6094	83.4464
LUFT	2006, 2007, 2008	Oconoluftee Visitor's Center, GRSM	35.5094	83.3050
MTLE	2006	Mount Leconte, GRSM	35.6564	83.4389
NFGA	2007	Newfound Gap, GRSM	35.6128	83.4411
OCOV	2006	Oconoluftee Overlook, GRSM	35.6072	83.4822
P02	2008	Plot 2, Road Prong Trail, GRSM	35.6100	83.4506
P03	2008	Plot 3, Road Prong Trail, GRSM	35.6106	83.4581
P30	2008	Plot 30, Andrew's Bald, GRSM	35.5383	83.4956
P48	2008	Plot 48, Fork Ridge Trail, GRSM	35.5872	83.4669
P49	2008	Plot 49, Fork Ridge Trail, GRSM	35.5858	83.4622
P50	2008	Plot 50, Fork Ridge Trail, GRSM	35.5850	83.4572
P51	2008	Plot 51, Fork Ridge Trail, GRSM	35.5850	83.4572
P56	2008	Plot 56, Appalachian Trail, GRSM	35.5917	83.4650
P57	2008	Plot 57, Appalachian Trail, GRSM	35.5961	83.4619
P58	2008	Plot 58, Appalachian Trail, GRSM	35.5981	83.4575
P59	2008	Plot 59, Appalachian Trail, GRSM	35.6011	83.4556
P60	2008	Plot 60, Appalachian Trail, GRSM	35.6044	83.4511
POGA	2006, 2007	Polls Gap, GRSM	35.5633	83.1614
PUKN	2006, 2007	Purchase Knob, GRSM	35.6314	83.1792
RIBA	2007, 2008	Richland Balsam, BLRI	35.3619	82.9889
SPFI	2008	Spruce Fir Nature Trail, GRSM	35.5956	83.4583
SPLA	2008	Sparks Lane, GRSM	35.6014	83.7950
SWHE	2008	Sweat Heifer Trailhead, GRSM	35.6208	83.4039
WAKN	2008	Waterrock Knob, BLRI	35.4594	83.1414

Table 6. Total mercury loads in juvenile songbird feathers collected in Great Smoky Mountains National Park in 2006. Mercury values ($\mu\text{g/kg}$, fw) are presented as mean $\pm$ SE (n). Banding locations are as follows: Indian Gap (INGA), Oconoluftee Overlook (OCOV), Clingmans Dome (CLDO), Mt. LeConte (MTLE), Great Smoky Mountains Institute at Tremont MAPS station (GSMI), Oconoluftee Visitors Center (LUFT), Polls Gap (POGA), and Purchase Knob MAPS station (PUKN).

Species	INGA	OCOV	CLDO	MTLE	GSMI	LUFT	POGA	PUKN	Grand Mean
Veery			1462.5 (1)						1462.5 (1)
Winter Wren	922 $\pm$ 233 (5)		438 (1)						841 $\pm$ 207 (6)
Yellow-breasted Chat								778 (1)	778 (1)
Louisiana Waterthrush					681 $\pm$ 123 (2)				681 $\pm$ 123 (2)
Canada Warbler	843 $\pm$ 216 (3)						481 $\pm$ 93 (4)	582 $\pm$ 123 (2)	624 $\pm$ 94 (9)
Eastern Towhee								613 $\pm$ 65 (2)	613 $\pm$ 65 (2)
Least Flycatcher								602 (1)	602.4 (1)
Brown Creeper		124 (1)	600 (1)	772 $\pm$ 194 (2)					567 $\pm$ 172 (4)
Dark-eyed Junco	728 $\pm$ 74 (20)	850 $\pm$ 181 (4)	450 $\pm$ 76 (4)	482 $\pm$ 80 (7)			470 $\pm$ 265 (3)	331 $\pm$ 37 (15)	557 $\pm$ 44 (53)
Golden-crowned Kinglet	468 $\pm$ 86 (4)		595 (1)	503 $\pm$ 37 (4)				521 (1)	500 $\pm$ 36 (10)
Black-throated Blue Warbler	710 (1)						424 (1)	450 $\pm$ 77 (10)	469 $\pm$ 68 (12)
Song Sparrow						821 (1)		437 $\pm$ 87 (15)	461 $\pm$ 85 (16)
Carolina Wren	267 (1)						719 (1)	428 $\pm$ 53 (2)	461 $\pm$ 97 (4)
Hooded Warbler							460 (1)		460 (1)
Gray Catbird						405 $\pm$ 44 (3)			405 $\pm$ 44 (3)
Ovenbird								333 $\pm$ 33 (7)	333 $\pm$ 33 (7)
Rose-breasted Grosbeak								250 $\pm$ 86 (2)	250 $\pm$ 86 (2)
Chestnut-sided Warbler							524 (1)	195 $\pm$ 39 (5)	250 $\pm$ 63 (6)
Common Yellowthroat								236 (1)	236 (1)
Eastern Phoebe								220 $\pm$ 33 (2)	220 $\pm$ 33 (2)
Downy Woodpecker								208 $\pm$ 16 (2)	208 $\pm$ 16 (2)
American Robin						189 (1)			189 (1)
Carolina Chickadee							120 $\pm$ 71 (2)	174 (1)	138 $\pm$ 45 (3)
Black-throated Green Warbler					124 (1)				124 (1)
All Species	722 $\pm$ 61 (34)	705 $\pm$ 202 (5)	612 $\pm$ 129 (8)	533 $\pm$ 56 (13)	495 $\pm$ 199 (3)	445 $\pm$ 106 (5)	439 $\pm$ 73 (13)	380 $\pm$ 27 (69)	557 $\pm$ 54 (150)

Table 7. Total mercury loads in songbird feathers collected in Great Smoky Mountains National Park in 2007. Mercury values are presented as mean $\pm$ SD ($\mu\text{g/kg}$, fw); sample sizes are in parentheses. Banding locations are as follows: Black Rock Trail (BKRT), Cades Cove (CACO), Coopers Gap (COGA), Eagle Creek (EACR), Fork Ridge Trail (FORK), Great Smoky Mountains Institute at Tremont MAPS station (GSMI), Newfound Gap (NFGA), Oconoluftee Visitors Center (LUFT), Polls Gap (POGA), Purchase Knob (PUKN), Richland Balsam (RIBA), and Waterrock Knob (WAKN). Various GRSM represents multiple locations within Great Smoky Mountains National Park.

Species	BKRT	CACO	COGA	EACR	FORK	GSMI	NFGA
Carolina Wren				3744 (1)			
Eastern Towhee							
Belted Kingfisher						994 (1)	
Winter Wren					903 $\pm$ 3 (2)		
Hooded Warbler				912 (1)			
Blue-gray Gnatcatcher							
Golden-crowned Kinglet							
Blackburnian Warbler							
Ruffed Grouse							
Barn Swallow		593 (1)					
Indigo Bunting		53.6 (1)					
Eastern Phoebe		509 $\pm$ 157 (5)					
Canada Warbler							
Carolina Chickadee							
Song Sparrow							
Overbird							
Dark-eyed Junco	297 $\pm$ 110 (6)		230 $\pm$ 68 (6)		172 $\pm$ 63 (5)		472 $\pm$ 264 (12)
Black-and-white Warbler	213 (1)						
Gray Catbird							
Yellow Warbler							
Field Sparrow		172 (1)					
Chestnut-sided Warbler							
American Crow							
Eastern Meadowlark		119 (1)					
House Finch							
Total	385 $\pm$ 303 (9)	387 $\pm$ 245 (9)	230 $\pm$ 68 (6)	2328 $\pm$ 1416 (2)	445 $\pm$ 304 (10)	994 (1)	472 $\pm$ 264 (12)

Table 7 continued.

Species	LUFT	POGA	PUKN	RIBA	WAKN	Various GRSM	Grand Mean
Carolina Wren	365 (1)						2054 ± 2389 (2)
Eastern Towhee			1413 (1)				1413 (1)
Belted Kingfisher							994 (1)
Winter Wren							903 ± 3 (2)
Hooded Warbler			777 (1)				844 ± 96 (2)
Blue-gray Gnatcatcher	738 ± 25 (2)						738 ± 25 (2)
Golden-crowned Kinglet				617 ± 878			638 ± 492 (8)
Blackburnian Warbler			585 (1)				585 (1)
Ruffed Grouse						573 ± 787 (3)	573 ± 787 (3)
Barn Swallow							593 (1)
Indigo Bunting			785 ± 899 (2)				541 ± 763 (3)
Eastern Phoebe							509 ± 157 (5)
Canada Warbler		714 (1)	654 (1)		308 (1)		449 ± 219 (3)
Carolina Chickadee	379 (1)						379 (1)
Song Sparrow	329 ± 115 (4)		368 ± 113 (5)				351 ± 116 (9)
Ovenbird			327 ± 40 (2)				327 ± 40 (2)
Dark-eyed Junco		252 ± 313 (9)		195 ± 69 (12)	298 ± 79 (6)	245 ± 149 (3)	283 ± 198 (71)
Black-and-white Warbler							213 (1)
Gray Catbird	190 ± 62 (3)						190 ± 62 (3)
Yellow Warbler	188 (1)						188 (1)
Field Sparrow							172 (1)
Chestnut-sided Warbler			156 ± 34 (4)				156 ± 34 (4)
American Crow	140 ± 32 (2)						140 ± 32 (2)
Eastern Meadowlark							119 (1)
House Finch	5 (1)						5 (1)
Total	313 ± 213 (14)	302 ± 313 (11)	389 ± 322 (29)	280 ± 334 (15)	299 ± 73 (7)	409 ± 538 (7)	393 ± 419 (135)

Table 8. Total mercury loads in songbird feathers collected in Great Smoky Mountains National Park in 2008. Mercury values are presented as mean $\pm$ SD ($\mu\text{g/kg}$, fw); sample sizes are in parentheses. Banding locations are as follows: Fork Ridge Trail (FORK), Great Smoky Mountains Institute at Tremont MAPS station (GSMI), Hazel Creek Campground (HACR), Plot 2 along Indian Gap Trail (P02), Plot 3 along Indian Gap Trail (P03), Plot 30 along Forney Ridge Trail (P30), Plot 48 along Fork Ridge Trail (P48), Plot 49 along Fork Ridge Trail (P49), Plot 50 along Fork Ridge Trail (P50), Plot 51 along Fork Ridge Trail (P51), Plot 56 along Fork Ridge Trail (P56), Plot 57 along Fork Ridge Trail (P57), Plot 58 along Fork Ridge Trail (P58), Plot 59 along Fork Ridge Trail (P59), Plot 60 along Fork Ridge Trail (P60), Richland Balsam (RIBA), Spruce Fir Nature Trail (SPFI), Sparks Lane in Cades Cove (SPLA), and Sweat Heifer Trail (SWHE).

Species	FORK	GSMI	HACR	P02	P03	P30	P48
Sharp-shinned Hawk							
Carolina Wren		1060 $\pm$ 233 (4)					
Louisiana Waterthrush		927 (1)					
Winter Wren							
Brown Creeper							
Golden-crowned Kinglet	817 $\pm$ 397 (2)					727 (1)	
Black-and-white Warbler			487 (1)				
Black-capped Chickadee							
Veery							
Slate-colored Junco	325 $\pm$ 18 (2)			584 $\pm$ 390 (2)	304 (1)	408 $\pm$ 240 (16)	319 $\pm$ 114 (3)
Eastern Phoebe			321 $\pm$ 185 (2)				
Total	571 $\pm$ 365 (4)	1033 $\pm$ 211 (5)	376 $\pm$ 162 (3)	584 $\pm$ 390 (2)	304 (1)	427 $\pm$ 245 (17)	319 $\pm$ 114 (3)

Table 8 continued.

Species	P49	P50	P51	P56	P57	P58	P59
Sharp-shinned Hawk							
Carolina Wren							
Louisiana Waterthrush							
Winter Wren						797 ± 225 (2)	
Brown Creeper							
Golden-crowned Kinglet					297 (1)		434 (1)
Black-and-white Warbler							
Black-capped Chickadee					337 ± 29 (2)		
Veery							
Slate-colored Junco	215 ± 67 (3)	226 ± 105 (3)	319 ± 38 (3)	327 ± 162 (3)	707 ± 432 (10)	454 ± 168 (2)	315 ± 89 (3)
Eastern Phoebe							
Total	215 ± 67 (3)	226 ± 105 (3)	319 ± 38 (3)	327 ± 162 (3)	618 ± 410 (13)	626 ± 256 (4)	344 ± 93 (4)

Table 8 continued.

Species	P60	RIBA	SPFI	SPLA	SWHE	Grand Mean
Sharp-shinned Hawk		1475 (1)				1475 (1)
Carolina Wren				826 ± 282 (3)		960 ± 263 (7)
Louisiana Waterthrush						927 (1)
Winter Wren		1015 (1)	804 (1)			853 ± 169 (4)
Brown Creeper		606 (1)	850 (1)			728 ± 173 (2)
Golden-crowned Kinglet		570 ± 281 (3)	1008 ± 910 (3)		588 (1)	701 ± 484 (12)
Black-and-white Warbler						487 (1)
Black-capped Chickadee		606 ± 441 (2)				471 ± 299 (4)
Veery		436 (1)				436 (1)
Slate-colored Junco	147 ± 18 (2)	401 ± 233 (16)	370 ± 99 (6)		387 ± 238 (18)	403 ± 257 (94)
Eastern Phoebe						321 ± 185 (2)
Total	147 ± 18 (2)	515 ± 328 (25)	627 ± 512 (11)	826 ± 282 (3)	398 ± 235 (19)	494 ± 333 (129)

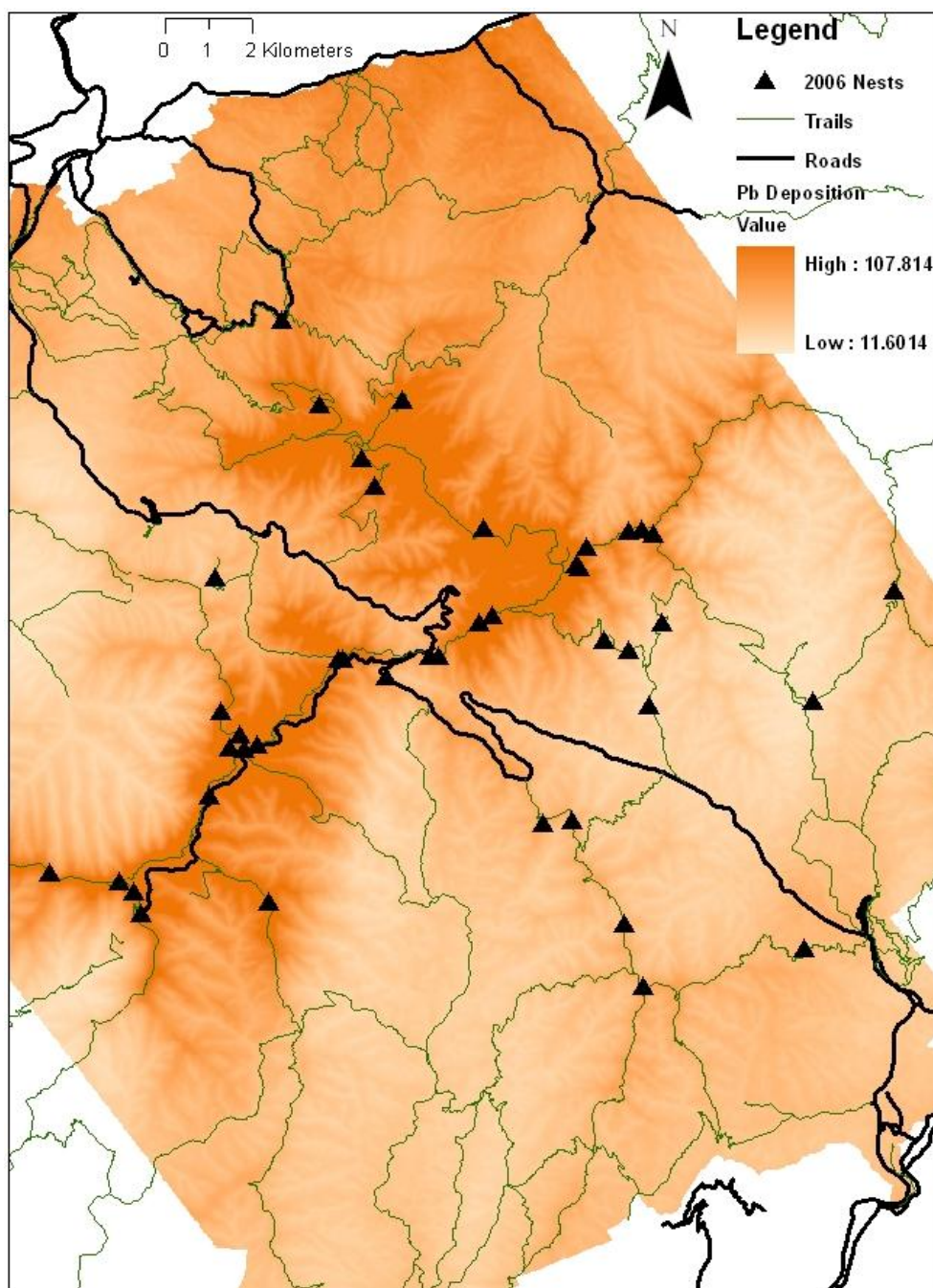


Figure 1. Distribution of Dark-eyed Junco nests ($n = 36$) in Great Smoky Mountains National Park whose eggs were collected and analyzed for calcium content. Lead deposition (mg Pb/kg) represents predicted Pb concentrations in the soil organic horizon in GRSM.

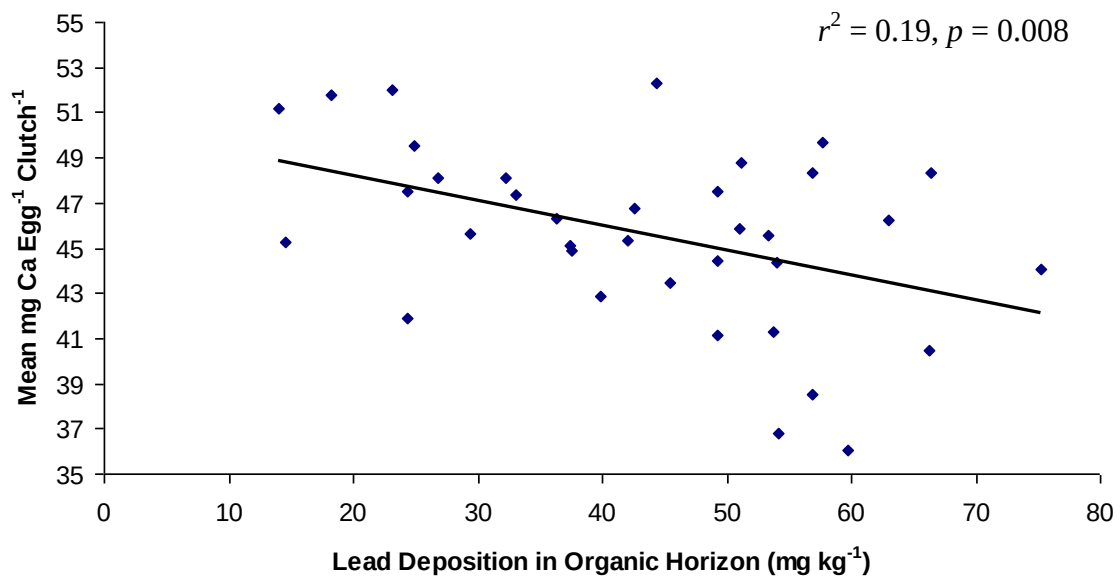


Figure 2. Linear regression of mean calcium content (mg) per egg per clutch ($n = 36$) in Dark-eyed Juncos on lead deposition mg Pb/kg organic horizon in GRSM.

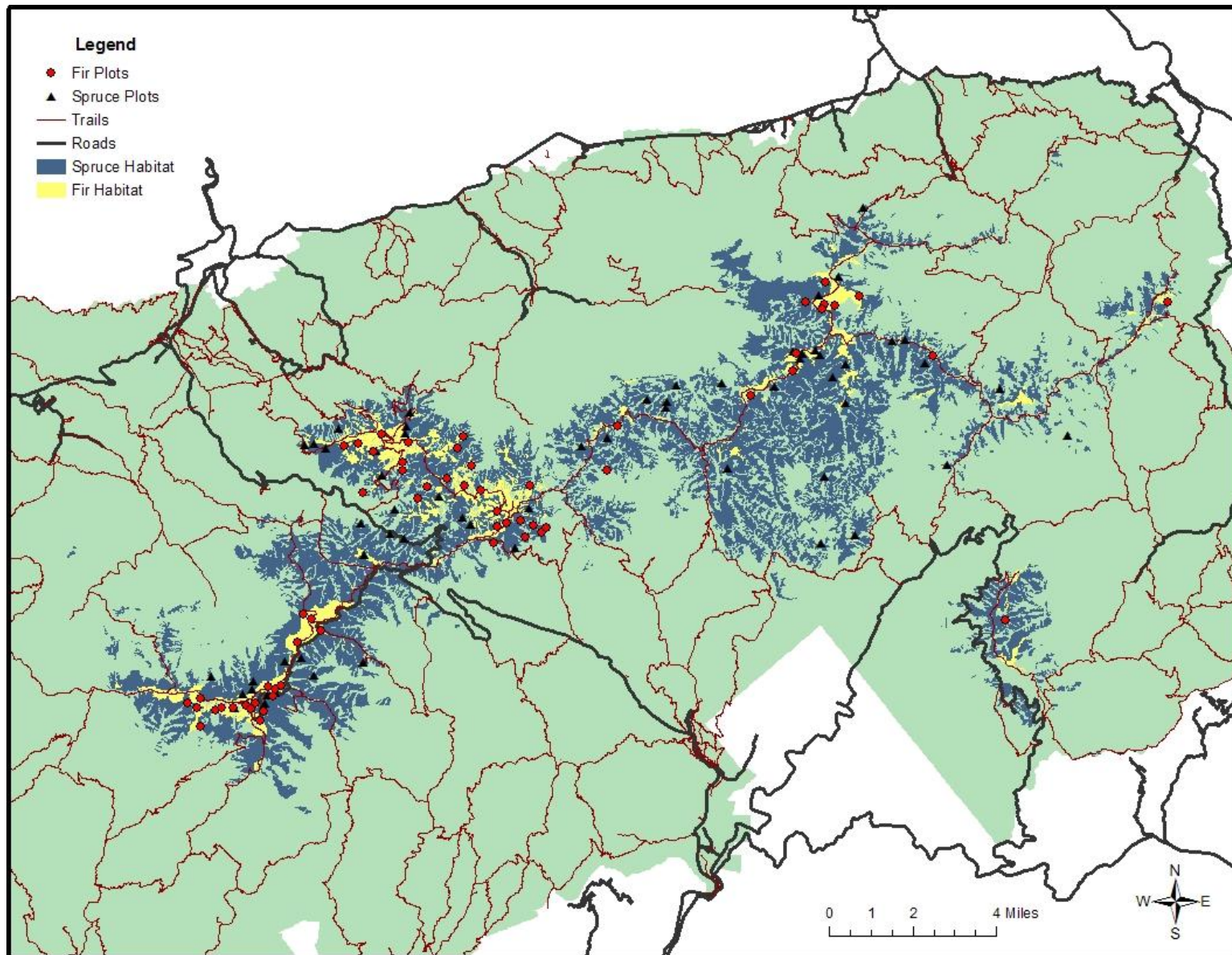


Figure 3. Snail plot locations from 2006 and 2007 within spruce and fir habitats in Great Smoky Mountains National Park (green extent).

Pb (mg/kg), independence model

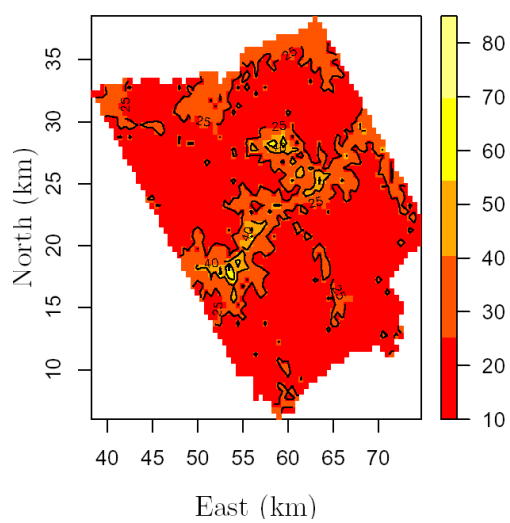


Figure 4. Maps of predicted Pb levels in Great Smoky Mountains National Park from Weathers et al. (2006) regression model.

Figure 5. Maps of predicted Pb levels in Great Smoky Mountains National Park by Webster et. al (in prep) based on data by Weathers et al. (2006). These models incorporate measures of spatial dependence and prediction error.

