Mercury Bioaccumulation in a Terrestrial Food Web of a Montane Forest

Christopher C. Rimmer[1], Eric K. Miller[2], Kent P. McFarland[1], Steven D. Faccio[1], Allan B. Strong[3], and Robert J. Taylor[4]

[1] Vermont Center for Ecostudies, P.O. Box 420, Norwich, VT 05055
[2] Ecosystems Research Group, Ltd., P.O. Box 1227, Norwich, VT 05055
[3] Rubenstein School of Natural Resources and Environment, Aiken Center, 81 Carriage drive, University of Vermont, Burlington, VT 05405
[4] Trace Element Research Laboratory, Texas A & M University, VMA Building, Room 107, Highway 60, College Station, TX 77843-4458

Abstract

We investigated mercury (Hg) concentrations in a terrestrial food web in high elevation forests in Vermont. Mercury concentrations increased from autotrophic organisms to herbivores < detritivores < omnivores < carnivores. Within the carnivores studied, raptors had higher blood mercury concentrations than their songbird prey. The Hg concentration in the blood of the study focal species Bicknell's thrush varied over the course of the summer in response to a diet shift related to changing food item availability. The montane food web is more detrital-based (with higher Hg concentrations) in early summer and more foliage-based (with lower Hg concentrations) during late summer. There were significant year effects in different ecosystem compartments indicating a possible connection between atmospheric Hg deposition, detrital-layer Hg concentrations, arthropod Hg concentrations, and passerine blood Hg levels.

Introduction

Methylmercury (MeHg), the bioavailable form of mercury (Hg), is a neurotoxin with well-documented, adverse impacts on natural systems and wildlife populations. Most investigations on Hg bioaccumulation and biomagnification have focused on freshwater aquatic ecosystems, where conditions promoting methylation are common and Hg concentrations in upper trophic level consumers may be high (e.g., Bank et al. 2005, 2007; Chen et al. 2005; Evers et al. 2005; Yates et al. 2005). Research has increasingly demonstrated that Hg impairs reproductive performance, lifetime productivity, growth and development, behavior, motor skills, and survivorship in aquatic birds and other wildlife (Wolfe et al. 1998; Evers 2004, 2008; Scheuhammer et al. 2007). Despite the recent documentation of elevated Hg exposure in terrestrial biota (summary in Driscoll et al. 2007), relatively little is known about pathways for Hg uptake and transfer in upland ecosystems, or about Hg risk thresholds for terrestrial organisms.

Trophic transfer of Hg in a strictly terrestrial food web has not been documented, although Cristol et al. (2008) showed Hg biomagnification in biota from a terrestrial habitat adjacent to a Hg-contaminated river in Virginia. Hg concentrations increased in known avian prey items (Orthoptera [grasshoppers] → Lepidoptera [moths or caterpillars] → Aranea [spiders]) to passerine birds. Nearly 50% of the Hg in spiders, which comprised 20-30% of diet in three focal songbird species, was in the form of bioavailable MeHg. Twelve of 13 avian species sampled had significantly higher Hg blood concentrations at the contaminated site than at uncontaminated reference sites. Cristol et al. (2008) concluded that aquatic Hg moved into and through the terrestrial food web, where avian consumption of predatory invertebrates increased the food chain length and caused Hg to biomagnify.

In montane areas of northeastern North America, anthropogenic Hg deposition from atmospheric sources is 2-5 times higher than in surrounding low elevation areas (Miller et al. 2005). Although mechanisms that drive methylation in montane forests are poorly understood, Hg has recently been documented to bioaccumulate in montane fauna of the Northeast (Bank et al. 2005, Rimmer et al. 2005, Evers and Duron 2008). In particular, Bicknell's thrush *(Catharus bicknelli)*, a Nearctic-Neotropical migratory songbird has been shown to exhibit elevated Hg blood and feather concentrations among all age and sex classes across its breeding range (Rimmer et al. 2005). This rare, range-restricted habitat specialist of montane forests is an avian species of high continental conservation concern (Rimmer et al. 2001, Rich et al. 2004). As a higher trophic level consumer, primarily of arthropods, Bicknell's thrush is a potentially valuable bioindicator of montane forest ecosystem health. Understanding of Hg burdens in this species and in trophic compartments of its food web could contribute to species-specific and ecosystem-based conservation planning.

To elucidate trophic transfer of Hg in montane forests, we sampled leaf litter and biota at a long-term study site in the northeastern U.S. Our goals were to examine Hg concentrations and their variability among compartments of a terrestrial food chain during the montane summer.

Study Area and Methods

Field sampling

As part of long-term demographic research on montane forest bird populations in the northeastern U.S., we investigated the bioaccumulation and trophic transfer of Hg on Stratton Mountain (43° 05' N, 72° 55' W) in southern Vermont. From late May through late July in 2004-2007, we sampled discrete compartments in the terrestrial food web, using an established study site between 1075-1180 m elevation. To reflect a range of trophic levels, we sampled leaf litter, foliage, folivorous and carnivorous arthropods, a terrestrial salamander, an insectivorous passerine bird, and two carnivorous raptors. Salamanders and birds were sampled across a study area of c. 25 ha between 1075-1180 m elevation, while we collected leaf litter, foliage and arthropod samples at two sites 50 m apart at 1100 m elevation. One site was situated on the northwest-facing edge of a 30-m wide ski slope, the second 50 m to the west in mature, closed-canopy, fir-dominated forest.

Avian sampling was conducted on a near-daily basis throughout the entire sampling period in each summer, typically between dawn and mid-morning and from late afternoon through dusk, weather permitting. Sampling of litter and other biota was conducted opportunistically in dry and relatively warm weather, both to maximize logistic efficiency and to take advantage of peak activity patterns of exothermic arthropods. Because inclement weather is frequent at high elevations, we were unable to sample litter, foliage and arthropods as frequently as planned.

Care was taken to ensure that samples were not contaminated, especially those that required manual handling (e.g., litter and foliage). We generally used latex gloves during sampling, and we cleaned sampling utensils with distilled water and/or 5% nitric acid. All samples were frozen within 2 hours of collection.

<u>Leaf litter</u>: At both sampling sites, we collected leaf litter and organic soil samples of ~250 cm^3 at a depth of 5-10 cm, using a small hand trowel that was wiped cleaned and rinsed with nitric acid and distilled water between individual sampling events. Care was taken not to include any portion of the underlying inorganic soil horizon. We collected three samples per site on 21 July 2004, 6 June 2006, and 15 June 2007; on 13 July 2007, we collected one sample at each site. Each sample was double-bagged in Ziploc® bags.

<u>Foliage</u>: We sampled whole leaves of three dominant deciduous tree species (paper birch [*Betula papyrifera* var. *cordifolia*], American mountain-ash [*Sorbus americana*], and pin cherry [*Prunus pennsylvanica*] and needles from the dominant conifer (balsam fir [*Abies balsamea*]), generally following methods outlined by Rea et al. (2002). Using hand pruners (wiped and cleaned with distilled water between each individual sampling event), we snipped the distal 20-30 cm of branch tips between 2-3 m height. On deciduous species, this yielded samples of 8-12 leaves, while coniferous branch tips generally contained 6-10 branchlets. We sampled and homogenized for analysis the previous 2-3 years of growth of fir needles on each branch, with the exception of three 2007 samples for which we separately clipped and analyzed needles grown in 2005, 2006 and 2007. For each species, we collected three replicates at both sampling sites on five dates: 21 July 2004, 8 and 28 June 2005, and 15 June

and 13 July 2007. All foliage samples were transferred immediately upon collection to Ziploc® plastic bags, double-bagged inside a second Ziploc® bag.

Arthropods: We sampled terrestrial and arboreal arthropods at both sites on six dates: 21 July 2004, 8 and 28 June 2005, 11 July 2006, and 15 June and 13 July 2007. We collected ground-dwelling arthropods (as well as a single sample of small gastropods) primarily through visual searches and gentle probing of the top leaf litter layer. Individuals were collected either with plastic forceps or a rubber aspirator, then transferred immediately to small plastic vials. For flying and arboreal arthropods, we used sweep nets, shook understory branches onto plastic protective sheets, or collected foliage-dwelling individuals with forceps or directly into storage vials. For small and medium-sized arthropods, we typically combined multiple individuals of a distinct taxon (e.g., ants, spiders, opiliones) into single storage vials. Prior to analysis, we identified each sample, whether consisting of a single or multiple individuals, to the lowest possible taxonomic level (usually order), using several references that included Borror and White (1970), Borror et al. (1981), and on-line sources such as BugGuide.Net (Iowa Sate University 2009). The very small masses of many individual arthropods (below detection limits for Hg determination) required lumping them by identifiable taxon for laboratory analyses. For all taxa for which we collected an adequate number of individuals for analysis, we archived at least one frozen reference sample.

Red-backed salamander: On 26 June 2006, we conducted active searches for red-backed salamanders (*Plethodon cinereus*) in forested habitat on Stratton Mountain by turning over objects (logs, rocks, etc) where salamanders often hide. All salamanders were captured by hand at 1000-1110 m elevation, placed in a moistened plastic bag, and measured (snout-to-vent, and total length). A tissue sample was collected from each individual by clipping a small (~ 5mm) portion of their tail tip using surgical scissors. Hg levels in salamander tail tips have been shown to provide a good correlation with whole body Hg burdens (D. Evers, personal comm.). Salamanders were then immediately released at their point of capture. All samples were immediately stored in Whirl-pak® sample bags and Ziploc® bags.

Birds: Using standard arrays of 6-m and 12-m, 36-mm mesh nylon mist nets throughout our study site, we captured individuals of our focal avian species, Bicknell's thrush, using both passive and broadcast elicitation methods. In the course of this netting, we incidentally captured individuals of two raptorial species, sharp-shinned hawk (*Accipiter striatus*) and northern saw-whet owl (*Aegolius acadicus*). Sharp-shinned hawks are predators on small passerines, and are known to regularly depredate Bicknell's thrush (Rimmer et al. 2001). Northern saw-whet owls primarily feed on small rodents, but they occasionally take passerine birds, including *Catharus* thrushes (Rasmussen et al. 2008) and are thus potential predators of Bicknell's Thrush. All captured birds were banded with uniquely numbered U.S. Fish and Wildlife Service aluminum leg bands, aged and sexed according to standard criteria (Pyle et al. 1997, Collier and Wallace 1988), and weighed prior to release. A series of morphometric measurements was also taken. From each individual of these three species, we collected a 30-50 µl blood sample from the cutaneous ulnar (brachial) vein in a 75 µl heparinized capillary tube, which was sealed on both ends with Crito-seal or Critocaps® and placed in a labeled glass 7 cc vacutainer. We sampled blood from all individuals upon their initial captures in each year, and we selectively collected subsequent blood samples from individuals captured one week or more after collection of their previous sample.

Laboratory Analyses

All samples were shipped in liquid nitrogen or ice to Texas A&M University Trace Element Research Laboratory (TERL) for analysis by element-specific cold vapor atomic absorption. We measured only total Hg in each compartment, rather than bioavailable MeHg. Although the ratio of MeHg to total Hg may vary temporally and geographically within and among taxa (Cristol et al. 2008, Evers and Duron 2008), total Hg concentrations are commonly used to indicate exposure. All results from TERL are presented in units of parts per million (ppm or ug/g) as wet weight (ww) for avian blood and dry weight (dw) for other compartments.

Statistical Analyses

We examined all Hg data for normality. Non-normal data were log-transformed prior to analysis. Descriptive statistics, linear regressions and ANOVA analyses were calculated with JMP 6.03 (SAS Institute). We also used General Linear Models (GLMs) in SYSTAT 12 (Systat Software 2008) to examine within-season and between-year effects in Hg data for each sampled compartment, using different combinations of potential interactions as terms in the model.

Our previous work showed that Hg blood concentrations of both individual birds and the sampled population significantly declined during the breeding season (Rimmer et al. 2005). We therefore modeled blood Hg levels for all thrushes sampled in more than one year using a GLM, in which the interaction between year (2004-2007) and date were used as terms in the model. Because the interaction was significant ($F_{3,98} = 5.126$, $p = 0.002$), we examined each year separately and found 2004 to be significantly different from 2005-2007. A GLM excluding 2004 was not significant for the year terrm ($F_{2,72} = 0.141$, $p = 0.869$). We then examined blood Hg in a GLM using sex, age (second-year and after second-year), date, and an interaction between sex and date as terms, with data pooled for 2005-2007 and repeated for 2004 alone. We included the sex-date interaction because females can depurate Hg through egg laying (e.g., Thompson 1996, Monteiro and Furness 2001, Evers et al. 2005), which occurs primarily during the first three weeks in June (Rimmer et al. 2001).

There were significant and clear discontinuities in the overall declining trend of Bicknell's thrush blood Hg during the season with blood levels rising rapidly from day 113 through day 158 and falling fairly rapidly from day 160 through day 165. This period was followed by a steady and slower rate of decline through day 206. For the purposes of statistical analysis, the period prior to day 165 was classified as "Early" and day 165 and afterward as "Late" season. A one-sided t-test was used to evaluate differences in blood Hg levels between Early and Late season.

Classification of invertebrate foraging guilds

We classified arthropod samples in three broad guilds (Detrital, Canopy, Varied) according to the base of their known or suspected trophic web. Detrital arthropods include detritivorous organisms living primarily in the bark of dead and downed trees, as well as in leaf litter and upper soil layers. Canopy dwellers include herbivorous taxa inhabiting most structural forest layers from the forest floor to the uppermost tree canopy. We considered Varied arthropods to be those that are primarily carnivorous and feed on either Canopy or Detrital organisms.

We further classified arthropods within each guild (primarily by order) as Carnivorous, Omnivorous, Herbivorous-Detrital, Herbivorous-Canopy, and Varied. Carnivores included arachnids (spiders and harvestmen) and blood-sucking Diptera. Herbivorous-Detrital included organisms that feed on plants and fungi in the detrital layer, while the Herbivorous-Canopy class included organisms that feed on above-ground plant structures (generally live plants). The Varied class captured organisms such as some Diptera that are typically considered omnivorous. For ants, our personal observations suggested that early season foraging occurs primarily in the detrital layer (i.e., Herbivorous-Detrital), while late season ants were more often seen feeding in the canopy (i.e., Herbivorous-Canopy). For other Hymenoptera (e.g., wasps, bees, sawflies), our field observations and literature searches suggested that all were Herbivorous-Canopy foragers.

Finally, we classified arthropods as early- or late-season based on the dates on which they were sampled in each year (1-27 June = early, 28 June – 21 July = late). Although little information exists on the dietary composition of Bicknell's thrush during its breeding period, the species is reported to be a "versatile" feeder in both microhabitat and behavior (Rimmer et al. 2001). Under the assumption that thrushes are opportunistic foragers, taking available prey in proportion to their abundance and ease of capture, we further assumed that our opportunistic sampling constituted an first-order proxy for foraging success, and that the within- and between-year composition of our arthropod samples reflected the prey items available to Bicknell's thrush. We therefore lumped all orders in our analyses of date and year effects on Hg concentrations, rather than comparing individual orders, which were subject to small sample sizes, high variance and disparities in total biomass or species composition among sampling events. This sample compositing should reflect the Hg signal in food items available to the focal species Bicknell's thrush.

Results

Overall, Hg concentrations showed a generally increasing trend by trophic level (Fig. 1, Appendix). Leaf litter deviated markedly from this pattern, with Hg levels elevated above those in any biotic compartment except sharp-shinned hawk, the top trophic level consumer in our samples (Fig. 1, Appendix). This is not unexpected as leaf litter has lost much of its original mass via decomposition while retaining most of its original Hg content in addition to binding additional Hg deposited via rain, snow, and canopy throughfall (Grigal 2003). Mean litter Hg concentrations differed among years ($F_{2,15} = 6.46$, $p = 0.01$), but not sampling location ($F_{1,15} = 0.02$, $p = 0.88$), being significantly higher in 2006 than in 2004 and 2007, which did not significantly differ. Although sampling dates were not uniform among years, their appeared to be no seasonal bioaccumulation of Hg in leaf litter, as evidenced by the relatively high mean value of 0.323 $\pm$ 0.09 SD on 6 June 2006 and the lack of significant difference in mean values between samples collected on 13 June and 15 July 2007 (0.228 $\pm$ 0.102 SD ppm [n = 6] and 0.292 $\pm$ 0.031 SD [n = 2], respectively; t = 0-1.836, df = 5.954, p = 0.12).

Hg concentrations in balsam fir branches with aggregated needle samples (2-3 years of growth) were higher than those in the three deciduous tree species, both individually and combined (Fig. 1, Appendix). Single-year needle samples consistently increased in Hg content with age at an annual rate of 0.0142 ug/g ($R^2 = 0.94$, $p < 0.0001$). Seasonally, deciduous leaves showed significantly increasing Hg levels with date ($F_{1,50} = 4.92$, $p = 0.03$), accumulating 0.00001 ug/g per day during the growing season. Aggregated fir needles showed no between-year or within-season temporal trend, but Hg tissue concentrations were significantly higher in fir branches sampled in the forest interior than on the ski area edge ($F_{1,17} = 2.69$, $p = 0.001$).

Hg concentrations in arthropods ranged widely but were lowest in herbivorous insects, highest in predatory taxa (Araneae [spiders], Neuroptera [lacewings], and Opiliones [harvestmen]; Fig. 1, Appendix). The single gastropod sample showed relatively high Hg burdens, while the Diptera sample was elevated in part by an outlier value of 0.982 ppm in a single bloodsucking tabanid (deer fly). Among the three habitat-based foraging guilds, Detrital and Varied arthropods had significantly higher Hg levels than Canopy foragers (ANOVA: $F_{2,173} = 10.42$, $p < 0.0001$), but were not significantly different from each other. Carnivorous, Omnivorous and Herbivorous-Detrital foraging classes showed significantly higher Hg concentrations than Varied or Herbivorous-Canopy arthropods (ANOVA: $F_{4,171} = 32.8$, $p < 0.001$). Although not significantly different, Hg in Carnivorous arthropods was higher than in Omnivorous foragers, which in turn had higher Hg levels than Herbivorous-Detrital arthropods.

We found no significant year effect in Hg levels within any of the six arthropod orders (Araneae, Opiliones, Coleoptera, Diptera, Hymenoptera, and Lepidoptera [larvae]) that yielded sufficient sample sizes for analysis among years. However, combining all arthropods from each sampling event yielded a marginally significant effect of year, with 2004 significantly lower than 2005-2007 (Tukey-Kramer HSD p=0.05, ANOVA p=0.06). This year effect is interpreted as a combination of differences in Hg concentrations and differences in orders represented in the samples from each year. Due to the opportunistic nature of the sampling, the different representation of orders probably represents a differential food-item availability between 2004 and 2005-2007. No within-season effects of date were found for either individual orders or all arthropods combined in 2005 or 2007, the two years in which early- and late-season sampling was conducted.

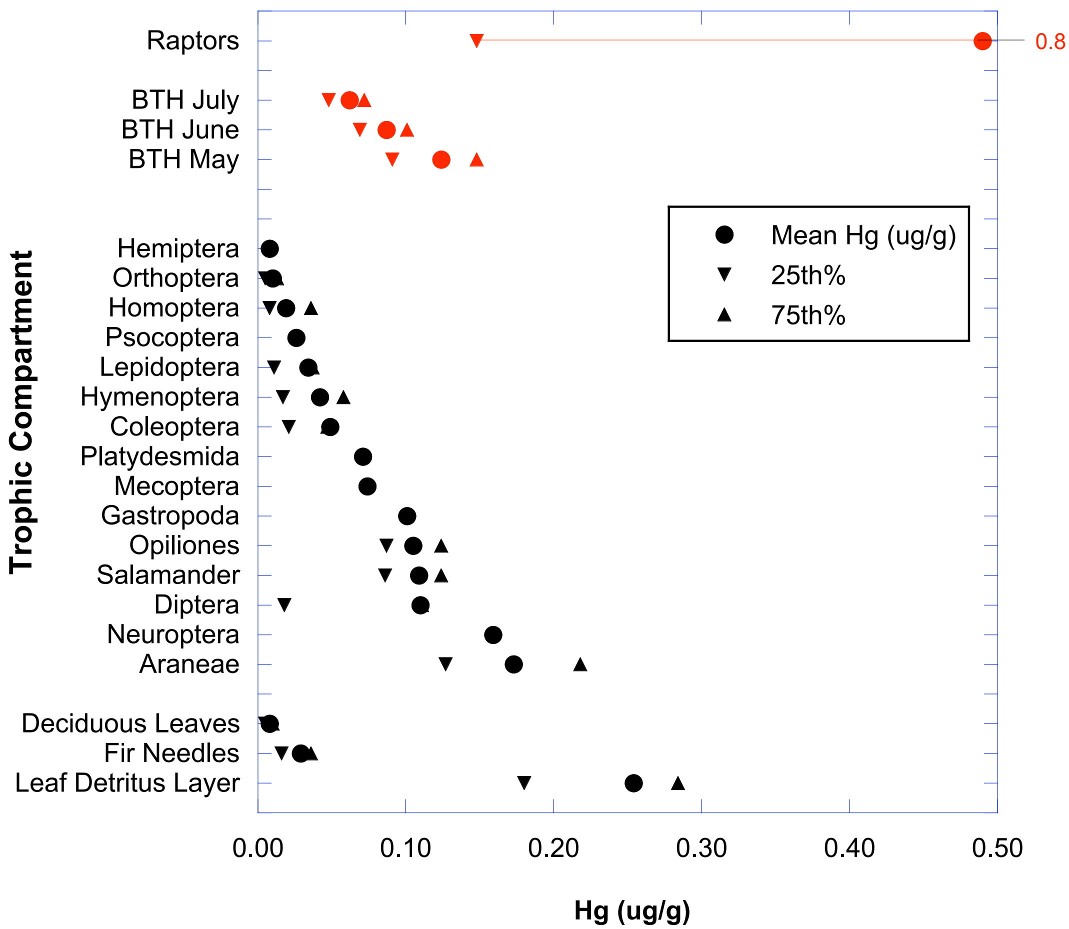


Figure 1. Mean, 25th, and 75th percentile Hg concentrations for leaf litter and biota sampled on Stratton Mountain, Vermont in 2004-2007.

Among the three foraging guilds, we found a significant temporal shift in the proportions of arthropods sampled, from Detrital (63% of total) dominant in early season samples to a more even distribution between Detrital (36%), Varied (33%), and Canopy (32%) foragers in late season samples ($\chi^2 = 15.16$, df = 2, $p = 0.0005$). Similarly, foraging subclasses showed a shift between early and late season samples, with Carnivorous (19% early, 14% late) and Herbivorous-Detrital arthropods (7% early, 5% late) declining in proportional abundance and Herbivorous-Canopy invertebrates (6% early, 16% late) increasing ($\chi^2 = 1.99$, df = 4, $p = 0.018$).

Among vertebrates, Hg burdens in red-backed salamanders were comparable to those in several invertebrate groups on which they reportedly prey, including Opiliones, Gastropoda, and Diptera (Fig 1; Appendix). For the 151 blood samples from adult Bicknell's thrushes, a marked year effect was evident, with Hg concentrations in 2004 (0.071+/-0.006 ug/g) significantly lower than in 2005-2007 (0.093+/-0.004 ug/g; ANOVA p=0.0002, Tukey-Kramer HSD p=0.05), which did not differ. Bicknell's thrush blood Hg concentrations showed no effects of sex or age classes in 2004 or 2005-2007, nor any interaction between sex and date. However, for the sample population, blood Hg concentrations showed a significantly decreasing linear trend with date across all years (r^2=0.36, p<0.0001, n=150). Although statistically significant as a linear trend over the season, the temporal pattern of Hg blood concentration was actually a rapid increase followed by a rapid decrease, followed by a more steady decline through the end of the season (Figure 2). Early season (prior to day 165) blood levels were significantly different from late season levels (p<0.00001, one-sided t-test). Blood Hg concentrations in the two predatory bird species, northern saw-whet owl and sharp-shinned hawk, were elevated above those of Bicknell's thrush, markedly so in the latter species (Fig. 1, Appendix).

Discussion

Although specific trophic relationships within the montane forest food web are not well documented, the Hg concentrations we report here appear to reflect biomagnification from lower to higher trophic levels. Although Hg levels within each compartment or biotic group did not invariably conform to known or suspected patterns of trophic transfer, the general progression was consistent with expectations: food web base (foliage < litter), herbivorous arthropods < detritivorous arthropods < predatory arthropods < insectivorous vertebrates < carnivorous vertebrates (Figure 1, Appendix). The congruence of year effects in leaf litter, arthropods and Bicknell's thrush blood further suggests the existence of dietary linkages across trophic compartments at this montane forest site.

Foliage and leaf litter

Our results conform to those of other studies that show high leaf litter Hg concentrations relative to those in live foliage (e.g., up to 60% greater), due to the accumulation of Hg over time, and the concentration of Hg relative to nutrients that leach and are translocated out of foliage during senescence (Lindberg 1996; Rea *et al*. 1996, 2002; Tyler, 2005). Hg foliar concentrations in the three deciduous species on Stratton were slightly higher than those reported from five hardwoods species at mid-elevations in north-central Vermont (Rea et al. 2002). Aggregated balsam fir needle Hg concentrations were higher than those of deciduous leaves; current-year needle Hg levels were almost identical, while those of 2- and 3-year old needles progressively increased and were higher than deciduous leaf Hg levels. This was an expected result, because our aggregated fir needle samples were composed of 3 years of growth, and thus accumulated Hg sequestration, while deciduous leaves reflected Hg uptake only since leaf-out 0.5-1.5 months prior to sampling (Grigal 2003).

Softwood-dominated leaf litter at Stratton Mountain showed relatively high Hg concentrations compared to those of most biotic compartments. Hg in litter is derived both from litterfall, rain, snow, and throughfall (Grigal 2003). Litter Hg concentrations reflect deposition, retention and release, but these mechanisms and their relationship to bioavailability of Hg in montane forest litter need further investigation. Demers et al. (2007) found that litter Hg accumulated during the growing season. Hall and St. Louis (2004) also reported that both MeHg and total Hg concentrations in softwood-dominated litterfall of Canadian boreal forests increased over time (800 days).

Arthropods

Although few published data exist on Hg concentrations of terrestrial arthropods, our data conform to those of others in which primary consumers (herbivores and detritivores) show lower Hg levels than secondary consumers (predatory species). Zheng et al (2008) studied three arthropods in a Hg-contaminated grassland of China and found Hg concentrations of 0.043 and 0.037 ug/g in two primary consumers (*Locusta* sp. and *Acrida* sp.) and "higher" (no value given) Hg concentrations in a secondary consumer, *Paratenodera sinensis*. Cristol et al (2008) sampled orthopterans, lepidopterans, and spiders in Virginia upland habitats adjacent to Hg-contaminated rivers and at uncontaminated reference sites. Mean Hg concentrations of all three orders were "negligible" at reference sites, and lower than values we obtained on Stratton Mountain, while Hg levels at contaminated sites were dramatically higher (spiders = 1.24 $\pm$ 1.47 ug/g, lepidopterans = 0.38 $\pm$ 2.08 ug/g, orthopterans = 0.31 $\pm$ 1.22 ug/g; Cristol et al. [2008]). In the Catskill region of New York, preliminary data, based on small sample sizes, suggested spiders have Hg levels 2-3 times higher than those of other arthropods (Evers and Duron 2008).

Red-backed salamander

Although the diet of red-backed salamanders in montane forests is not well known, Burton (1976) found that the species preyed primarily on mites, spiders, snails, and numerous insect families at the Hubbard Brook Experimental Forest in New Hampshire. The relatively high levels of Hg in our salamander samples suggest that they are feeding at a high trophic level within the invertebrate community, or that their preferred prey accumulate relatively high amounts of Hg due to micro-habitat preferences, soil strata, or other variables. Red-backed salamanders live and forage in moist soils, often near stream edges where total sediment Hg and MeHg levels are highest (Morel et al. 1998). Although our sampling effort was limited, we found salamanders only along stream edges on our study site. Since they rarely move away from these moist micro-habitats, their prey may consist of a disproportionate number of invertebrates found only along stream edges. Salamander densities in the montane forest appear to be quite low, possibly due to predominantly shallow, acidic soils. These soils have been shown to disrupt sodium balance in red-backed salamanders, which are rarely found on soils with a pH $\leq$3.7 (Frisbie and Wyman 1991).

Birds

Blood Hg concentrations in Bicknell's thrush (breeding season average 0.088+/-0.003 ug/g) were lower than previously reported in this species on Stratton Mountain (0.12 $\pm$ 0.04 ug/g; Rimmer et al. 2005). There was an initial increase in blood Hg level from 0.1 ug/g to 0.13 ug/g during the first weeks on the breeding ground (days 113-158, Figure 2). Blood Hg levels then declined rapidly from days 160-165 after which a more steady but lower rate of decline persisted through the last samples on day 206 (Figure 2). Although data on dietary composition of this species are scant, owing to the difficulty of direct observation and sampling known food items, our data strongly suggest that a seasonal shift in diet accounts for the initial increase followed by declining Hg blood levels during June and July.

As a long-distance migrant, Bicknell's thrush spends 7-8 months per year away from its northeastern U.S. breeding sites (Rimmer et al. 2001). Previous research has shown that blood Hg concentrations of thrushes sampled in January and February on their Caribbean winter grounds averaged 2-3 times higher than in birds sampled on breeding sites (Rimmer et al. 2005). An exponential decay model was constructed to estimate the carry-over effects of Hg burdens obtained on the wintering grounds on Hg observations during the breeding season. Although there appears to be no information on the half-life of MeHg or total Hg in passerine blood, published data exist for non-molting adults of three primarily aquatic birds. The half-life of blood MeHg is 31.5-63 days for great skua (*Catharacta skua*; Bearhop et al. 2000), 40-60 days for Cory's shearwater (*Calonectris diomedea*; Monteiro and Furness 2001), and 74 days for mallard (*Anas platyrynchos*; Heinz and Hoffman 2004). Because Bicknell's thrushes are much smaller than these three species, with higher basal metabolism and presumably lower absolute rates of Hg ingestion, we conservatively estimate 30 days as a probable half-life of MeHg in Bicknell's thrush blood. Furthermore, due to the higher than normal metabolic demands of migration, the loss of Hg obtained on the wintering grounds is likely further accelerated during transit from wintering to breeding grounds. Nearly all individuals depart their wintering sites for northward migration before 1 May and arrive at Vermont breeding sites before 1 June (authors' unpubl. data). Using parameters of blood Hg at time of wintering ground departure equal to 2.5 times the average breeding ground concentration, a 30-day wintering-breeding ground transit time, and a 30-day half-life, an exponential decay model successfully predicted the initial breeding-ground blood Hg observations (0.101 ppm, n=4 on day 113; Figure 2).

There was a significant difference ($P<0.00001$, 1-sided t-test) between Early and Late season blood Hg concentrations in Bicknell's thrush. Birds return to their breeding grounds during the very early stages of leaf-out and prior to the emergence of most folivorous arthropods. At this time, spiders are relatively numerous (pers. obs.), and we suspect that this group constitutes a significant portion of the species' diet. As a primarily ground-foraging species, Bicknell's thrush likely feed disproportionately on spiders, harvestmen, and ants early in the growing season. Snails may also be taken by females to supplement calcium mobilization for egg production. As new coniferous and deciduous foliage emerges during June, Bicknell's thrush likely shift to a higher proportion of folivorous arthropods, such as adult and larval ledpidopterans, hymenopterans (sawflies and ichneumons), and hemipterans. The change in food item availability and lower Hg burdens in late-season potential prey items likely account for the drop in thrush blood concentrations between early and late summer on the breeding grounds (Figures 2 and 3).

Arthropod data corroborate an apparent seasonal diet shift by Bicknell's thrush along the Hg contamination spectrum of potential prey items (Figure 3). Arthropods showed declines in abundance of Carnivorous and Herbivorous-Detrital classes (those highest in Hg) between early and late season samples, and a preponderance of lower trophic order organisms (with lower Hg concentrations) later in the season (Figure 3).

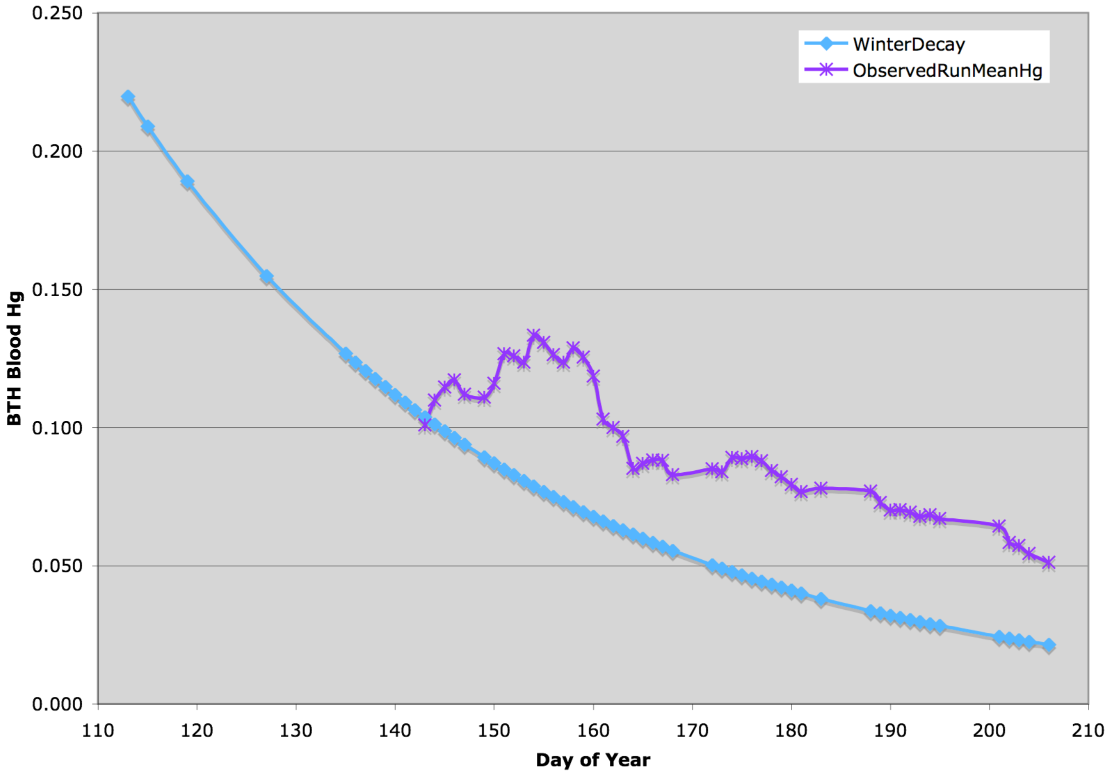
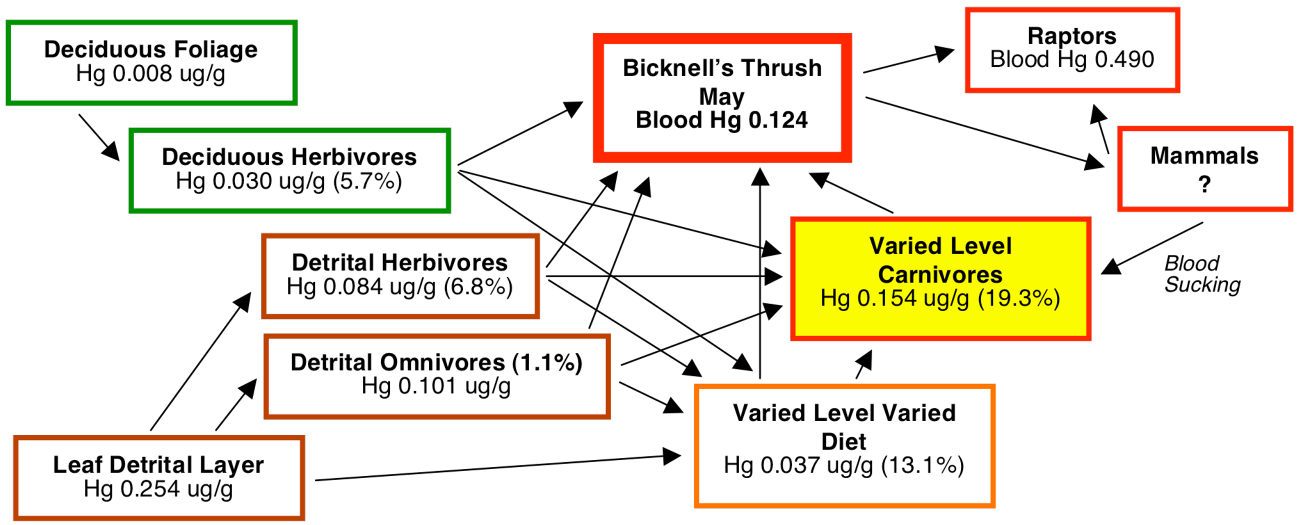


Figure 2. Exponential decay model (light blue) of the dissipation of wintering ground Hg burden and observations (purple) of Hg blood concentrations on the breeding grounds in Bicknell's thrush. Due to the fluctuations in number of birds captured and sampled daily, the observed blood levels are presented as the 10-day moving average.

The Hg level in Bicknell's thrush blood at the end of the breeding season is 1.6 times the level of residual wintering-ground Hg burden predicted by the exponential decay model. This suggests that Hg in the breeding ground diet provides a significant component of the total Hg burden of the birds during the breeding season. Still, the Hg burden carried from the wintering grounds is substantial. Further study is warranted into sources of Hg in the winter diet of Bicknell's thrush and other co-occurring migratory birds in the same wintering areas.

The two predatory bird species, sharp-shinned hawk and northern saw-whet owl, showed elevated blood Hg from Bicknell's thrush and red-backed salamanders (Fig. 1, Appendix). Reflecting its exclusive diet of small songbirds, including Bicknell's thrush, sharp-shinned hawk blood Hg was expected to be higher, and was likely accounted for by trophic biomagnification. The order of magnitude increase above Bicknell's thrush was surprising; however, variance was large. Northern saw-whet owls showed Hg blood concentrations that likely reflected this species' dietary specialization on small mammals, most of which feed on seeds and vegetation. In contrast to Bicknell's thrush, one individual sharp-shinned hawk captured on 5 June and again on 13 July 2006 had nearly identical blood Hg on the two dates, 0.967 and 0.975 ppm, respectively. Hawks are unlikely to switch prey items within a summer, and this finding reinforces the likelihood that seasonal declines in thrush blood Hg signal a dietary shift from carnivorous to herbivorous arthropods.

Early Season Food Web



Late Season Food Web

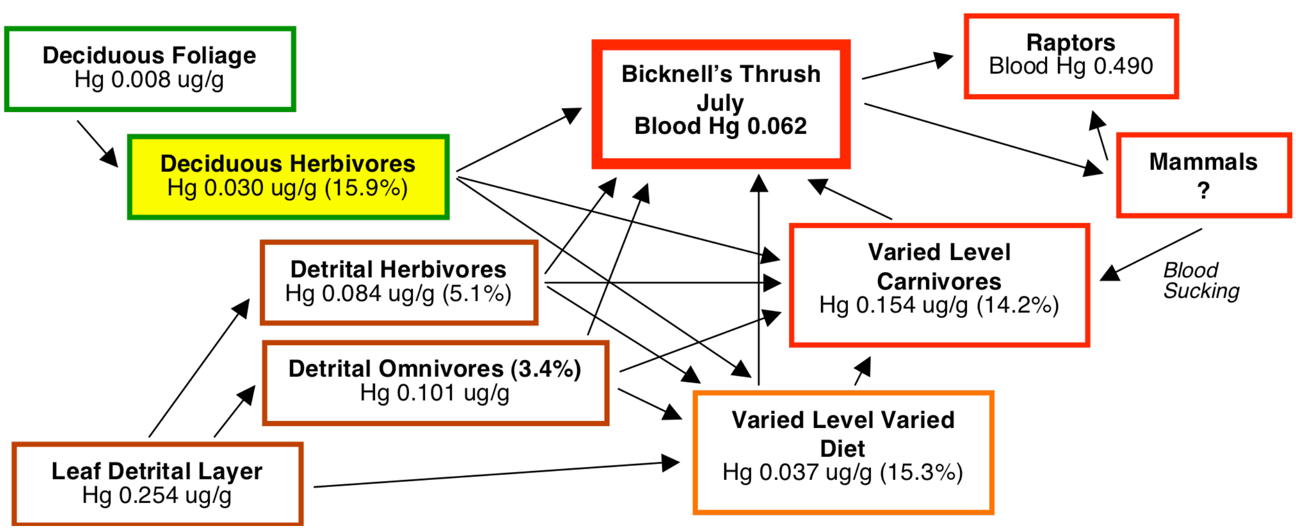


Figure 3. Shifts in food web structure from early to late summer in a montane ecosystem. The relative abundance of different arthropod feeding guilds (as percents in parentheses) and each compartment's mean Hg level are indicated. A detrital-based food web dominates the early season, while a canopy-based food web increases in importance during the late summer season. Bicknell's Thrush (red) is the focal species in this study.

Year effects in Hg concentrations

Significantly lower Hg concentrations in three sampled compartments (litter, arthropods, Bicknell's thrush) during 2004 versus 2005-2007 provide compelling evidence for dietary linkages and trophic transfer in the terrestrial montane forest community. Bioavailability of Hg, as reflected through uptake by Bicknell's thrush, has been shown to correlate to modeled atmospheric deposition patterns (Rimmer et al. 2005). Our results further corroborate this link, in that nearby (Underhill, VT) Hg deposition data were relatively lower from 1999-2003 (mean 9.3 $ug/m^2/year$) and relatively higher from 2004-2007 (mean 11.6 $ug/m^2/year$) (E. Miller, manuscript in preparation). As the early season food items are dependent on the detrital-based food web, there is likely at least a 1-year lag between deposition changes and changes in the Hg burdens of the detrital-based food web. This shift from a lower to higher mercury deposition regime could have accounted for the lower Hg levels on Stratton Mountain in 2004 and relatively higher levels from 2005-2007.

<u>Summary</u> – Overall, Hg concentrations showed a pattern of biomagnification at successive trophic levels in the montane forest food web (Fig. 1, Appendix 1). The within-season changes in Bicknell's thrush blood Hg levels were consistent with a diet switch from more Hg-rich detrital-based prey abundant in the early summer food-web to prey relatively lower in Hg content that were more abundant in the foliage-based food web of mid to late summer (Figures 2 and 3). The significant and consistent year effect among the trophic compartments of litter, arthropods and thrush blood strongly suggests that avian dietary differences are reflected in blood concentrations. These observations provide clear evidence that Hg bioaccumulates and biomagnifies in the montane forest biotic community.

Acknowledgments

We gratefully acknowledge funding support from the U.S. Environmental Protection Agency through the University of Vermont for this study. Our ongoing avian research on Stratton Mountain was supported by the Stratton Mountain Resort, Thomas Marshall Foundation, Vermont Monitoring Cooperative, and friends of the Vermont Center for Ecostudies. We thank the many dedicated field biologists who assisted with collection of these data under frequently difficult conditions. We are indebted to staff of the Texas A&M Trace Element Research Laboratory for conducting all aspects of the mercury analyses. Jason Townsend provided constructive reviews of an early draft.

Literature Cited

Bank, M. S., C. S. Loftin, and R. E. Jung. 2005. Mercury bioaccumulation in northern two-lined salamanders from streams in the northeastern United States. Ecotoxicology 14:181–191.

Bank, M. S. , J. Crocker, B. Connery, and A. Amirbahman. 2007. Mercury bioaccumulation in green frog *(Rana clamitans)* and bullfrog *(Rana catesbeiana)* tadpoles from Acadia National Park, Maine, USA. Environmental Toxicology and Chemistry 26:118-125.

Bennett, R. S., J. B. French, Jr., R. Rossmann, and R. Haebler. 2009. Dietary toxicity and tissue accumulation of methylmercury in American kestrels. Arch. Environ Contam Toxicol 56:149-156.

Borror, D. J. and R. E. White. 1970. A field guide to the insects of America north of Mexico. Houghton Mifflin, Boston, MA.

Boror, A. J., D. M. De Long, and C. A. Triplehorn. 1981. An introduction to the study of insects. Saunders College Publishing, Philadelphia, PA.

Burton, T.M. 1976. An analysis of the feeding ecology of salamanders of the Hubbard Brook Experimental Forest, New Hampshire. Journal of Herpetology 10:187-204.

Chen, C. Y., R. S. Stemberger, N. C. Kamman, B. M. Mayes, and C. L. Folt. 2005. Patterns of Hg bioaccumulation and transfer in aquatic food webs across multi-lake studies in the northeast US. Ecotoxicology 14:135-147.

Collier, B. and G. E. Wallace. 1988. Aging *Catharus* thrushes by rectrix shape. Journal of Field Ornithology 60: 230–240.

Demers, J. D., C. T. Driscoll, T. J. Fahey, and J. B. Yavitt. 2007. Mercury cycling in litter and soil in different forest types in the Adirondack region, New York, USA. Ecological Applications 17:1341-1351.

Driscoll, C. T., Y-J. Han, C. Y. Chen, D. C. Evers, K. F. Lambert, T. M. Holsen, N. C. Kamman, and R. K. Munson. 2007. Mercury contamination in forest and freshwater ecosystems in the northeastern United States. BioScience 57:17-28.

Ericksen, J. A., M. S. Gustin, D. E. Schorran, D. W. Johnson, S. E. Lindberg, and J. S. Coleman. 2003. Accumulation of atmospheric mercury in forest foliage. Atmospheric Environment 37:1613-1622.

Evers, D. C. and M. Duron. 2008. Assessing the availability of methylmercury in terrestrial breeding birds of New York and Pennsylvania, 2005-2006. BRI Report 2008-15.

Evers D.C., O. P. Lane, L. Savoy, and W. Goodale. 2004. Assessing the impacts of methylmercury on piscivorous wildlife using a wildlife criterion value based on the Common Loon, 1998–2003. Gorham (ME): Maine Department of Environmental Protection, BioDiversity Research Institute. BRI Report 2004-05.

Evers D. C., N. M. Burgess, L. Champoux, B. Hoskins,A. Major, W. M. Goodale, R. J. Taylor, R. Poppenga, and T. Daigle. 2005. Patterns and interpretation of mercury exposure in freshwater avian communities in northeastern North America. Ecotoxicology 14: 193–221.

Evers, D .C., L. J. Savoy, C. R. DeSorbo, D. E. Yates, W. Hanson, K. M. Taylor, L. S. Siegel, J. H. Cooley, Jr., M. S. Bank, A. Major, K. Munney, B. Mower, H. S. Vogel, N. Schoch, M. Pokras, M. W. Goodale, and J. Fair. 2008. Adverse effects from environmental mercury loads on breeding common loons. Ecotoxicology 17:69-81.

Frisbie, M. P. and R. L. Wyman. 1991. The effects of soil pH on sodium balance in the red-backed salamander and three other terrestrial salamanders. Physiological Zoology 64:1050-1068.

Hall, B. D. and V. L. St. Louis. 2004. Methylmercury and total mercury in plant litter decomposing in upland forests and flooded landscapes. Environmental Science and Technology 38:5010-5021.

Hammerschmidt, C. R. and W. F. Fitzgerald. 2005. Methylmercury in mosquitoes related to atmospheric mercury deposition and contamination. Environmental Science and Technology 39:3034-3039.

Iowa State University. 2009. BugGuide.Net. Iowa State University Entomology Department, Ames, IA. http://www.bugguide.net

Lindberg, S. E. 1996. Forests and the global biogeochemical cycle of mercury: the importance of understanding air/vegetation exchange processes. Pp. 359-380 *in* Baeyens, W., R. Ebinghaus, and O. Vasiliev (Eds.), Global and regional mercury cycles: sources, fluxes, and mass balances. Kluwer Academic Publishers, Dordrecht, Netherlands.

Miller, E. K., A. VanArsdale, J. G. Keeler, A. Chalmers, L. Poissant, N. C. Kamman, and R. Brulotte. 2005. Estimation and mapping of wet and dry mercury deposition across northeastern North America. Ecotoxicology 14:53-70.

Monteiro, L. R. and L. W. Furness. 2001. Kinetics, dose-response, and excretion of methylmercury in free-living adult Cory's shearwaters. Environmental Science and Technology 35:739-746.

Morel, F. M. M., A. M. L. Kraepiel, and M. Amyot. 1998. The chemical cycle and bioaccumulation of mercury. Ann. Rev. Ecol. Syst. 29, 543–66.

Rasmussen, J. L., S. G. Sealy and R. J. Cannings. 2008. Northern Saw-whet Owl *(Aegolius acadicus)*. The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://www.bna.birds.cornell.edu/bna/species/042

Rea, A. W., S. E. Lindberg, T. Scherbatskoy, and G. J. Keeler. 2002. Mercury accumulation in foliage over time in two northern mixed hardwood forests. Water Air and Soil ollution 133:49-67.

Rich, T. D., C. J. Beardmore, H. Berlanga, P. J. Blancher, M. S. W. Bradstreet, G. S. Butcher, D. W. Demarest, E. H. Dunn, W. C. Hunter, E. F. Iñigo-Elias, J. A. Kennedy, A. M. Martell, A. O. Panjabi, D. N. Pashley, K. V. Rosenberg, C. M. Rustay, J. S. Wendt, and T. C. Will. 2004. Partners in Flight North American Landbird Conservation Plan. Cornell Lab of Ornithology. Ithaca, NY.

Rimmer, C.C., K.P. McFarland, W.G. Ellison, and J.E. Goetz. 2001. Bicknell's Thrush *(Catharus bicknelli)*. *In* The Birds of North America, No. 592 (A. Poole & F. Gill, eds.). The Birds of North America, Inc., Philadelphia, PA.

Rimmer, C.C., K.P. McFarland, D.C. Evers, E.K. Miller, Y. Aubry, D. Busby, and R.J. Taylor. 2005. Mercury concentrations in Bicknell's Thrush and other insectivorous passerines in montane forests of northeastern North America. Ecotoxicology 14:223-240.

Scheuhammer, A.M., Meyer, M.W., Sandheinrich, M.B. and Murray, M.W. 2007. Effects of environmental methylmercury on the health of wild birds, mammals, and fish. Ambio 36:12-19.

Sheehan, K. D., I. J. Fernandez, J. S. Kahl, and A. Amirbahman. 2006. Litterfall mercury in two forested watersheds at Acadia National Park, Maine, USA. Water, Air, and Soil Pollution 170: 249–265.

Systat Software, Inc. 2008. SYSTAT 12. Systat Software, Inc., Chicago, IL. http://www.systat.com/SystatProducts.aspx.

Wolfe, M., Schwarzbach, F.S. and Sulaiman, R.A. 1998. Effects of mercury on wildlife: a comprehensive review. Environmental Toxicology and Chemistry 17: 146-60.

Yates, D. E., D.T. Mayack, K. Munney, D. C. Evers, A. Major, T. Kaur, and R. J. Taylor. 2005. Mercury levels in mink *(Mustela vison)* and river otter *(Lontra canadensis)* from northeastern North America. Ecotoxicology 14:263-274.

Zheng, D.-M., Q.-C. Wang, Z.-S. Zhang, N. Zheng, and X.-W. Zhang. 2008. Bioaccumulation of total and methyl mercury by arthropods. Bull. Environ. Contam. Toxicol. 81:95-100.

Appendix.

Means, standard deviations, and ranges of Hg concentrations (ug/g) in leaf litter and biotic compartments sampled on Stratton Mountain, Vermont in June and July of 2004-2007.

Compartment	Mean	SD	Range	N
Leaf litter (all years)	0.254	0.091	0.141-0.492	20
Leaf litter (2004)	0.199	0.04	0.141-0.259	6
Leaf litter (2006)	0.323	0.09	0.254-0.492	6
Leaf litter (2007)	0.217	0.058	0.149-0.314	7
Deciduous leaves (all species)	0.009	0.004	0.003-0.02	44
Paper birch	0.007	0.003	0.002-0.13	25
Mountain ash	0.009	0.005	0.005-0.22	26
Pin cherry	0.007	0.003	0.003-0.01	6
Balsam fir branches	0.029	0.014	0.009-0.058	22
Balsam fir needles (new)	0.006	0.001	0.005-0.008	4
Balsam fir needles (1-year)	0.019	0.002	0.018-0.021	4
Balsam fir needles (2-year)	0.035	0.02	0.034-0.037	3
Araneae (spiders)	0.173	0.081	0.02-0.334	19
Coleoptera (beetles)	0.048	0.067	0.004-0.391	36
Diptera (flies)	0.11	0.17	0.002-0.984	50
Gastropoda (snails)	0.101			1
Hemiptera (true bugs)	0.008	0.002	0.006-0.009	2
Heteroptera (assassin bugs)	0.016	0.17	0.004-0.04	4
Hymenoptera (ants, wasps, sawflies)	0.04	0.033	0.004-0.12	20
Lepidoptera (adult)	0.05	0.07	0.006-0.13	3
Lepidoptera (larvae)	0.29	0.28	0.007-0.108	13
Mecoptera (scorpionflies)	0.074			1
Neuroptera (lacewings)	0.159			1
Opiliones (harvestmen)	0.105	0.029	0.056-0.142	7
Orthoptera (grasshoppers)	0.01	0.005	0.003-0.014	4
Platydesmida (millipede)	0.071			1
Psocoptera (barkflies)	0.026			1
Red-backed salamander	0.11	0.02	0.085-0.131	4
Bicknell's thrush (early June 2004)	0.091	0.04	0.051-0.199	13
Bicknell's thrush (late June 2004)	0.063	0.022	0.035-0.107	12
Bicknell's thrush (early July 2004)	0.072	0.028	0.055-0.105	3
Bicknell's thrush (late July 2004)	0.03	0.012	0.014-0.046	12
Bicknell's thrush (May 2005-07)	0.123	0.047	0.05-0.267	35
Bicknell's thrush (early June 2005-07)	0.098	0.031	0.046-0.201	37
Bicknell's thrush (late June 2005-07)	0.083	0.022	0.046-0.132	30
Bicknell's thrush (early July 2005-07)	0.065	0.014	0.046-0.095	25
Northern saw-whet owl	0.164	0.059	0.107-0.251	6
Sharp-shinned hawk	0.989	0.501	0.393-1.62	4