Introduction

Researchers involved in studies that involve the capture and blood sampling of wildlife have a responsibility to maximize the information that they obtain from these efforts whenever possible. During a study examining the sero-prevalence of Eastern Equine Encephalitis virus (EEEV) in Maine songbirds we obtained blood samples for serum analysis for exposure to EEEV. We used the retained packed red blood cell (PCV) to compare Hg signals to whole blood (WB) obtained from the same individual. We focused on Northern Waterthrush (Parkesia noveboracensis) because they have been recognized as indicators for environmental Mercury (Hg) contamination and existing data suggests exposure across the life cycle for this species (Jackson et al. 2015). We also included several paired samples from Gray Catbirds (Dumetella carolinensis), Veeries (Catharus fuscescens), and Ovenbirds Seurus aurocapilla) to correlate WB Hg with PCV Hg.

The analysis of bird tissues, such as blood, can address a range of questions related to wildlife health including body condition, disease monitoring and ecology, toxicology, population genetics, and evolution. Researchers should continue to maximize the use of all blood components through collaboration and research networks.

Study Sites and Methods

Field Methods and Sampling. We captured songbirds using mist nets during a long-term migration monitoring study at River Point Bird Observatory in Falmouth, ME. During the Fall of 2015 and the spring of 2016 we obtained two blood samples from individual Northern Waterthrushes. A subset of birds were sampled opportunistically on days throughout each season. Blood was collected from the brachial vein following puncture with a 27 gauge needle using non-nylar wrapped capillary tubes. One sample was spun for 5 min. at 3000rpm in a Zipocrit centrifuge, the tube was scored and broken at the line of PVC and blood serum separation and the serum was ejected from the capillary tube into a small BD container with serum separator. The conserved PCV and the second, whole blood, were then frozen for total mercury analysis in the lab. We banded and released all birds after recording standard morphometric data. This research was conducted in accordance with federal and state permits.

Total Hg Analysis. All blood samples were analyzed at BRI’s Toxicology Laboratory in Portland, Maine. Samples were placed into quartz sample boats, weighed, and analyzed for total Hg using thermal decomposition technique with an automated direct Hg analyzer (DMAT 80, Milestone Incorporated, USA) using the US EPA Method 7473 (US EPA 2007). At the start of each analysis run we included two samples each of two standard reference materials (Dorm-4 and Dolt-5), five methods blanks, and two sample blanks. Every 20 samples a duplicate was analyzed. At the end of each analysis run we included two samples each of two standard reference materials (Dorm-4 and Dolt-4), 4 method blanks, and one sample blank. Mercury results were reported in parts per million (μg/g) wet weight.

Results

We observed a high correlation (r²=0.9758, R=0.478, R²=0.95552) between the Hg present in WB relative to the Hg present in PCV (Figure 1). Data show that Hg is present in red blood cells such that removal of the blood serum results in nearly double the concentration of Hg in PCV compared to WB. These data suggest that it may be acceptable to use total Hg in PCV values as a relatable measure of total Hg in WB

We detected levels of Hg in Northern Waterthrush blood in both migration seasons (Figure 2) with a higher exposure and higher variance observed in birds captured during the fall (two-way t-test, p=0.020). Of the 11 Northern Waterthrushes captured in the fall, two had WB Hg above impact level (≥0.7 mg/kg) (Jackson et al. 2011), and two others were above 0.65 mg/kg (Jackson et al. 2011).

Discussion

Monitoring environmental toxins using indicator species is a key first step toward understanding the ecological impact of contaminants.

Data from this study shows a high correlation between Hg in WB and PCV in songbirds. Importantly, the % PCV (hematocrit) varies in songbirds due to factors that include hydration level, migratory or breeding status, etc. Therefore, measuring Hg in PCV or the %PCV in whole blood samples may offer a refinement in method and increase the utility of samples obtained during research studies.

Specifically, in 108 Northern Waterthrush blood samples acquired in Belize, Mexico, Canada and Maine across all seasons NPCV ranged from 37.8% to 57.0%, mean = 46.1% +/- 3.7%. (R. Holberton, P Keenan unpublished data). This suggests that measuring Hg in PCV may offer an improved measure of Hg than simply measuring Hg WB. One approach approach might be to use a correction to standardize Hg WB values to a 50% PCV level to compensate for variation in %PCV and improve the reliability of these two analysis methods.

An important consideration from a toxicity perspective is how variation in %PCV might lead to acute toxicity during periods of physiological stress such as dehydration due to migratory or breeding activities. Birds are known to metabolize tissues that may have bound Hg during migration or fasting periods (Seawage et al. 2016). This could exacerbate the impacts of Hg exposure that occur during other life stages and lead to acute toxicity.

This work is a very small contribution that advances efforts to broaden the methods available to researchers to maximize the utility of their capture and sampling efforts.

Future Recommendations

Working toward a better understanding of the role of ecoxins on wildlife health will involve collaborations between disease ecologists, ecotoxicologists, natural historians, parasitologists and many others. The continued advancement of methods to assess wildlife health offers opportunities for rich collaborations. Furthermore, with regard to birds, several monitoring networks exist that can readily support collaborative efforts. On interesting idea to measure stress indicated aging due to contaminant exposure might be to correlate telomere shortening across a mercury exposure gradient. There is a lot more to do.

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Introduction
Understanding the behavior and movements of wildlife is critical for the effective conservation of migratory populations. Northern Saw-whet Owls (Aegolius acadicus) nest in boreal forests in North America and many individuals migrate south for the winter. Saw-whet Owls are considered abundant, but their nocturnal habits make it difficult to estimate population sizes, measure population change, or describe migratory patterns.

Saw-whet Owls are present in Maine year-round, and large numbers migrate through the state each fall. They are known to migrate over large bodies of water, including the Great Lakes and Atlantic Ocean, although the frequency of such cross-water migrations is unknown. Recent observations of Saw-whet Owls on Maine coastal islands during fall migration, and evidence that they travel great distances over water, suggest that at least a subset of the state’s migratory population may be using a coastal or offshore migration route in the Gulf of Maine.

Study Sites and Methods
Owl captures and banding. Northern Saw-whet Owls were captured at 14 sites in coastal Maine during four fall migration seasons (2010-2013; Figure 1). At each site, we used mist nets (1-4 nets) and audio playback of the Northern Saw-whet Owl territorial call to capture owls, according to standardized methodologies (www.projectowlet.org). Nets were generally opened during civil twilight and operated for six hours per night, weather permitting. We checked nets every 30-45 minutes, extracted owls, banded birds and collected standard body condition and morphometric data.

Modeling approach. To examine environmental variables affecting owl captures per night at each site, we used maximum likelihood estimation for generalized linear mixed models using a Poisson distribution (a common distribution used to fit count data) in package “lme4” for R (Bates et al. 2012). A base model, including only station hours and the random variables of site, date, and year was compared to five other models containing models of weather, habitat, and topographic predictor variables. Model fit was assessed using Akaike’s Information Criterion (AIC), and the best-fit model was used to examine the significance of relationships between predictor variables and owl capture rates (Table 1).

Weather data were collected for each night of captures from local airports (Figure 1), and averaged for 6pm-midnight. We also examined several topographic and microhabitat variables, as well as habitat characteristics within a 5-km radius of each capture site (Ferre & Anderson 2013). Due to high levels of correlation between the 14 habitat variables we examined, we conducted a principal components analysis of the correlations (RMP 8.0) to represent habitat variation between sites in generalized linear models. The first principal component represented variation between open water and disturbed/developed habitats, and the second described a gradient between coastal habitats and upland forests/wetlands (Figure 2).

Figure 1. Saw-whet owl captures at six sites located in coastal Maine. Symbols represent the average number of captures per hour that each station was open for the 2010-2013 fall migration seasons.

Results
Saw-whet Owl captures were negatively affected by high moonlight (Figure 3). The best-fit generalized linear mixed model included 10 variables that were significant predictors for captures (Table 1). All of these variables were statistically significant (p<0.05); the most important predictors were station hours, moon phase, average ceiling height (e.g., cloud cover), forest canopy height, distance of the site to the nearest coastline, and the principal component habitat variables. Saw-whet Owl captures were positively correlated with the number of hours spent each night attempting to capture them; average cloud ceiling height; average north-south wind vectors; nets located in road cuts/field edges; distance to the nearest coastline; open water habitats; and non-coastal habitats (including boreal forests, upland mixed forests, and freshwater wetlands). Captures were negatively correlated with the fraction of the moon’s face illuminated; average temperature; a taller forest canopy in the vicinity of nets; coastal and inland capture locations; perpendicular distance from the generalized northeast-southwest bearing of the state’s coastline (e.g., 1,110M coastline in Figure 1), more disturbed areas (including areas with more roads, agriculture, and grass/shrub habitats); and coastal habitats (cliff and rock, coastal scrub-herb, and salt marsh).

Discussion
A variety of weather and site variables appear to affect Saw-whet Owl stopover choices during fall migration. Owls are most likely to be captured at the edges of boreal forests and other upland forest habitats, on nights with cooler average temperatures, north winds, and clear skies (but not too much moonlight, which is hypothesized to make them more vulnerable to predation; Speicher et al. 2011). Migration activity is highly variable between irruptive and non-irruptive migration years, as has been noted elsewhere (Figure 3; Whalen and Watts 2002).

Saw-whet Owl migration stopover activity also appears to vary based on the topography of the Maine coastline. Owl captures tend to be more frequent on island sites, sites with large amounts of open water, and along the southwest-to-northeast vector of the Maine coastline. However, capture rates decrease with proximity to actual coastlines and in areas with high proportions of coastal scrub habitat. We suggest that this seemingly contradictory pattern could be generated by two factors:

(1) Like many other avian migrants, Saw-whet Owls tend to track the Maine coastline and use it as a directional guide during migration.

(2) However, Saw-whets are not attracted to coastal habitats per se (preferring more heavily forested areas).

We expect that the majority of the variation in capture rates in this study is due to real effects of topography, habitat quality, and weather on Northern Saw-whet Owl migratory decision making, though many other factors influence stopover habitat use. Coastal and offshore migration by Saw-whet Owls has been widely regarded in the literature as an irregular phenomenon. However, at least along the Maine coast, it appears to be a major migration route for this species. This study is the first to document the variables affecting saw-whet migratory stopovers in Maine, as well as detailing a previously unknown coastal and offshore migration route for Saw-whet Owls in the Gulf of Maine.