Stable Isotopes from Museum Specimens May Provide Evidence of Long-Term Change in the Trophic Ecology of a Migratory Aerial Insectivore

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Identifying the mechanisms of ecological change is challenging in the absence of long-term data, but stable isotope ratios of museum specimen tissues may provide a record of diet and habitat change through time. Aerial insectivores are experiencing the steepest population declines of any avian guild in North America and one hypothesis for these population declines is a reduction in the availability of prey. If reduced prey availability is due to an overall reduction in insect abundance, we might also expect populations of higher trophic level insects to have declined most due to their greater sensitivity to a variety of disturbance types. Because nitrogen isotope ratios (δ15N) tend to increase with trophic-level, while δ13C generally increases with agricultural intensification, we used δ15N and δ13C values of bird tissues grown in winter (claw) and during breeding (feathers) from museum specimens spanning 1880–2005, and contemporary samples from breeding birds (2011–2013) to test for diet change in a migratory nocturnal aerial insectivore, Eastern Whip-poor-will (Antrostomus vociferus) breeding in Ontario, Canada. To test if environmental baselines have changed as a result of synthetic N fertilizer use, habitat conversion or climate, we also sampled δ15N values of three potential prey species collected from across the same geographic region and time period. Over the past 100 years, we found a significant decline in δ15N in tissues grown on both the breeding and wintering grounds. Prey species did not show a corresponding temporal trend in δ15N values, but our power to detect such a trend was limited due to higher sample variance. Amongst contemporary bird samples, δ15N values did not vary with sex or breeding site, but nestlings had lower δ15N values than adults. These results are consistent with the hypothesis that aerial insectivore populations are declining due to changes in abundance of higher trophic-level prey, but we caution that museum-based stable isotope studies of terrestrial food chains will require new approaches to assessing baseline change. Once addressed, an ability to decode the historical record locked inside museum collections could enhance our understanding of ecological change and inform conservation decisions.

Keywords: Antrostomus vociferus, diet change, museum collections, nightjar, nitrogen fertilizer, population declines, trophic-level, Whip-poor-will
INTRODUCTION

Birds have acted as sentinels of environmental threats since canaries were first taken into coalmines. Concurrent population declines among bird species with shared life history traits can help us identify the risks associated with global climate change (Wikelski and Tertitski, 2016). In North America, many of the bird species currently experiencing the steepest population declines are aerial insectivores that migrate long distances (Blancher et al., 2007; Nebel et al., 2010; North American Bird Conservation Initiative Canada, 2012; Smith et al., 2015). Since these species occupy a variety of breeding and wintering habitats, loss of any single habitat type cannot explain the similarities in population trajectories (Smith et al., 2015; Michel et al., 2016). In contrast, all of these species share a diet dominated by the adult life-stages of insects, and have foraging behaviors that are particularly energetically demanding and dependent on weather conditions conducive to insect flight. These similarities in prey choice and behavior suggest that insect availability could be the common factor limiting their populations (Nebel et al., 2010; Smith et al., 2015).

Long-term data on insect abundances in the Americas are sparse, but large reductions in insect abundance have been documented for parts of Europe (Conrad et al., 2006; Hallmann et al., 2017). If overall insect abundances are decreasing, we could expect populations of higher trophic-level insects to decline most quickly (Didham et al., 1998) because these species would be most sensitive to reductions in prey availability at lower trophic levels within their food webs (Pimm and Lawton, 1977; Purvis et al., 2000; Hamer et al., 2015). Consequently, birds that rely on insect prey could be forced to feed at lower trophic levels. Because N and C isotope ratios in the body tissues of consumers reflect isotope ratios in their diet, stable isotope ratios can be used to detect shifts in diet and trophic level. Nitrogen isotope ratios in consumers (δ15N) are influenced by diet and nutritional status, but on average are ~3% higher than those of their prey due to the lighter isotope of nitrogen (14N) being preferentially excreted in urea (Peterson and Fry, 1987; Sears et al., 2008). In contrast, C isotope ratios (δ13C) are relatively conserved across trophic levels, but vary depending on the photosynthetic pathways and the degree of water stress experienced at the base of the food web (Peterson and Fry, 1987; Still and Powell, 2010; Basu et al., 2015).

For many species, the isotope ratios of museum specimen tissues may provide the only record of dietary change over historic time scales. While initially applied to bone collagen to identify shifts in prehistoric human diets (Ambrose, 1986), this technique has also been applied to animal bones and keratinized tissues to explore changes in marine and terrestrial food webs (Thompson et al., 1995; Fox-Dobbs et al., 2007; Farmer and Leonard, 2011; Crowley et al., 2012; Wiley et al., 2013; Moreno et al., 2016) and linkages between them (Darinmore and Reimchen, 2002; Chamberlain et al., 2005; Blight et al., 2015; Matsubayashi et al., 2015). Shifts in diet between types of mammal carrion (marine, terrestrial herbivores, and grain-fed livestock) by California Condors (Gymnogyps californianus; Chamberlain et al., 2005) and from vertebrate herbivores and toward plant materials by Brown Bears (Ursus arctos; Matsubayashi et al., 2015) have been detected by comparing both the δ15N and δ13C values of museum specimen tissues from the predators and potential prey types during different time periods. Like bone, both feather and fur keratins retain the isotopic signatures from the time of growth and remain chemically inert in museum collections, however they have the advantage of being easily sampled from living specimens as well (Mizutani et al., 1990; Hobson, 1999). Indeed, timeseries of feathers including samples from both living birds and museum collections have been used to link population declines in an endangered alcid, Marbled Murrelet (Brachyramphus marmoratus) to reductions in the relative quantity of high trophic-level fish in their diet over the twentieth century (Becker and Beissinger, 2006; Norris et al., 2007; Gutowsky et al., 2009).

Interpretation of stable isotope ratios remains challenging because it relies on knowing the isotopic signatures of the relevant producers in an ecosystem (Ohkouchi et al., 2015). In marine systems, sampling of the phytoplankton or primary consumers, like forage fish, have been used to estimate baseline isotope values for particular food chains (Blight et al., 2015). In terrestrial communities, plant foliage forms the base of many food chains. Foliage isotope values vary across spatial and temporal scales depending on climate, disturbance, and nutrient availability (Amundson et al., 2003; Craine et al., 2009; Pardo and Nadelhofer, 2010; Popa-Lisseau et al., 2015; Moreno et al., 2016; Taki et al., 2017) and with physiological differences between plant species, including variation in photosynthetic pathways (Basu et al., 2015; Courty et al., 2015; Taki et al., 2017) and mycorrhizal associations (Evans, 2001; Craine et al., 2009; Hynson et al., 2016). At longer time scales changes in atmospheric baselines, climate, and habitat succession can all cause progressive change in foliage isotopic signatures (Evans and Belnap, 1999; Fox-Dobbs et al., 2007; Wang et al., 2007; Taki et al., 2017). Atmospheric CO2 is becoming increasingly depleted due to the burning of fossil fuels (Keeling, 1979). Because atmospheric CO2 is the sole source of carbon integrated into tissues of producers, we can correct for this change over time (Verburg, 2007). Temperature and rainfall can influence fractionation of carbon between the atmosphere and plant tissues, but the direction of change differs depending on the photosynthetic pathway involved (Basu et al., 2015) and this variation is ~1/10th that of the differences in fractionation between C3 plants than for C4 plants (Peterson and Fry, 1987). Successional changes in mean foliage δ13C values are therefore most likely to represent changes in community composition (Taki et al., 2017). In contrast to carbon, the nitrogen integrated into plant tissues can come from a wide range of sources. To become accessible to plants, nitrogen must first either be fixed naturally by lightning or bacteria, or fixed synthetically and applied as fertilizers. Once integrated into plant or animal tissues, nitrogen is also recycled in the soil by fungal and bacterial decomposers. Synthetic fertilizer has a δ15N value near 0‰ (equal to nitrogen in its atmospheric N2 gas form), while other sources of fixed nitrogen can be enriched or depleted to varying degrees. An ice core sampled in Greenland and lake sediments throughout North America show evidence of declining δ15N values in -NO3 in the atmosphere that has been...
linked to the use of synthetic fertilizers (Holtgrieve et al., 2011; Felix and Elliott, 2013), but long-term studies of trophic ecology in marine systems have not found evidence of ecosystem-wide change in $\delta^{15}$N values (Thompson et al., 1995; Blight et al., 2015). Whether the quantity of synthetically-fixed nitrogen deposited directly from the atmosphere (Li et al., 2016), relative to the nitrogen being fixed naturally and recycled within terrestrial ecosystems, has systematically shifted baseline nitrogen isotope ratios is still unknown. Disturbance initially increases $\delta^{15}$N values of plant foliage and then forest succession results in a subsequent decline, likely due to both a decreasing surplus of nitrogen and increasing dependence on mycorrhizal associations (Wang et al., 2007). All of these factors can influence the stable isotope signatures of organisms near the base of a food web, and therefore also how we interpret the signatures found at higher trophic levels.

Using Eastern Whip-poor-wills (Antrostomus vociferus; hereafter “whip-poor-wills”) as a case study, we explore the utility of museum collections and stable isotope analysis for detecting diet change in long-distance migratory birds reliant on aerial insect prey. Some of the sharpest population declines for avian aerial insectivores have occurred in northeastern North America (Nebel et al., 2010; Smith et al., 2015; Michel et al., 2016). Amongst these, Eastern Whip-poor-will are visual predators that rely on twilight or moonlight to detect prey, which are then captured during short bursts of flight from a perch on the ground or a branch. As a result, low-altitude flying nocturnal and crepuscular moths (~60%) and beetles (~40%) dominate their diet (Garlapow, 2007; Cink et al., 2017). Beetle taxa are more likely to belong to higher trophic levels than moths (Bennett and Hobson, 2009), and the proportion of beetles relative to other insect groups found in swift (Chaetura spp.) guano accumulated in chimneys has declined in recent decades (Nocera et al., 2012; Pomfret et al., 2014). Whip-poor-wills breed throughout much of eastern North America and winter in the Florida panhandle southwards throughout most of Central America. During breeding, they prefer open-canopy forest habitats and forest edges, where moonlight penetrates the forest canopy and facilitates foraging. Little is known about their winter habitat preferences (Cink et al., 2017). Populations breeding in the northern portion of this range have exhibited declines of ~3.5% per year in recent decades (COSEWIC, 2009) and current distribution and abundance appears associated with insect abundance even in otherwise suitable habitats (English et al., 2017b). We compare temporal trends in $\delta^{15}$N and $\delta^{13}$C values of tissues grown during the breeding and non-breeding seasons with those in potential insect prey over the past 130 years. If beetle population declines are limiting food availability and/or diet quality for whip-poor-wills, this could be reflected in a decline in the $\delta^{15}$N values of whip-poor-will tissues but not in the tissues of their herbivorous insect prey. If a change observed in bird tissues is solely the result of changing baseline $\delta^{15}$N values of continental ecosystems, either due to synthetic nitrogen deposition or large-scale shifts in climate or habitat, we expect a decline in $\delta^{15}$N values over time with a similar slope to that observed in bird tissues to be common also to the prey insects sampled.

**MATERIALS AND METHODS**

**Tissue Selection**

We determined age and sex of individual birds based on plumage characteristics and chose tissues to sample that reflected conditions during different times of year. Starting with flight feathers, whip-poor-will begin molt 1–2 months after arriving on the breeding grounds, often while still incubating or rearing young (personal observation). Because feather molt is energetically costly, both in terms of reduced flight capability and keratin tissue production (Echeverry-Galvis and Hau, 2013), most individuals initiate molt toward the end of their breeding season. This means that isotope signatures in these first feathers in the molt sequence should represent one of the most energetically demanding stages in the annual cycle. Both the primary flight feathers and smaller primary coverts are replaced concurrently, and in sequence starting with the innermost feather. Juvenile flight feathers grow while chicks are still being fed by their parents, and are retained until molted during their first breeding season. Juvenile-grown primary coverts have distinctive buff-colored tips, so all adult birds captured (or collected, in the case of museum specimens) during the breeding season can be classified as either 1-year-old or as older adult birds (Pyle, 2001). Primary covert feathers from 1-year-olds will have stable isotope values that reflect their diet during their nestling period, while older adults will have feathers that reflect their diet during the current or previous breeding season, depending whether they were sampled before or after molt was initiated. Both the carbon and nitrogen isotope ratios of feathers have been shown to depend on diet during the month immediately prior to tissue growth (Bearhop et al., 2002). At all ages, male whip-poor-will are easily distinguished from females because they have bright white outer tail feathers. Female tails are all gray, brown, and beige.

Whip-poor-wills are long-distance migrants that spend more than half of their annual cycle on their non-breeding grounds (Cink et al., 2017). Fortunately, avian claws grow relatively slowly and can retain a winter-dominated stable isotope signature after arrival on the breeding grounds (Bearhop et al., 2003; Mazerolle and Hobson, 2005; Fraser et al., 2008; Ethier et al., 2010). The central toe on whip-poor-will feet bears a pectinate claw, meaning that it is edged with a comb-like structure with 5–9 wide barbs (Supplemental Figure 1). This comb-like portion of the claw appears to grow from the base of the claw at a rate of about one barb per month (personal observation), thus the outer barbs likely have stable isotope signatures that reflect their diet at least 5 months prior to capture.

**Study Area and Contemporary Sample Collection**

We focused our sample collection on the province of Ontario, Canada due to evidence of a ~50% decrease in the probability of detecting whip-poor-wills over recent decades (Cadman et al., 2007). To establish how contemporary carbon and nitrogen isotope values vary with the age and sex of birds, we sampled primary coverts and claw tissue from individuals captured between 5 May and 25 July in 2011–2013 at three
breeding sites situated across the species’ range in Ontario: Rainy River district (48° 52′N 94° 01′-08′W), Norfolk County (42° 42′N 80° 21′-28′W), in Frontenac County (44° 28′-34′N 76° 20′-25′W). The Rainy River sites consisted of a 40,000-hectare mosaic of agriculture, poplar (Populus sp.), coniferous forests, logged areas, and wetlands. The Norfolk County site was St. Williams Conservation Reserve, which consists of two forest patches totaling 1035 hectares of pine-oak sand barrens and pine reforestation in a zone of intensive agriculture. The Frontenac County site was Queen’s University Biological Station (QUBS), which consists of over 3200 hectares of deciduous forest and abandoned farmland in various stages of succession, both with scattered small rock barrens. Across the whole province both afforestation of abandoned farmland and deforestation for timber harvesting, agriculture and urban development have continued throughout the twentieth century that together result in an overall loss of unimproved farmland and an increase in woodland (Cadman et al., 2007). A concurrent geolocator study established that winter locations of whip-poor-wills from Rainy River and Frontenac overlap throughout southern Mexico and Central America, but that Norfolk birds may winter further north in Mexico or the southern United States (English et al., 2017a). Due to this low connectivity between breeding and wintering locations, we do not assess spatial variation in winter grown tissues because all samples were collected on the breeding grounds.

At each field site, we captured adult male whip-poor-wills at night using song playback and mist nets. We targeted females and juveniles at QUBS, where we were able to capture them when they were incubating, or brooding nestlings, by placing a soft mesh fishnet over them. We identified all birds as male or female, aged them (juvenile, 1-year-old or older adult), recorded weight and wing length, and marked them with a numeric aluminum leg-band issued by the Canadian Wildlife Service. To sample tissue with isotopic signatures representing diet during the breeding season, we clipped one of the first four primary coverts. We sampled from amongst the first few feathers in the molt sequence to limit the seasonal variation in the timing of feather growth between individuals. In total, we collected 51 feathers grown by adults and 15 grown by juveniles (Table 1). To sample a tissue with isotopic signatures representing diet during the non-breeding season, we collected the outermost 2–4 claw barbs from 100 adults. Of these, 24% were 1-year-olds, which had just completed their first full migratory cycle. Capture and tissue sampling of birds followed the safety protocols of the Ornithological Council (Gaunt et al., 1997) and was approved by Environment Canada (sub-permit #10759 AG) and the Simon Fraser University Animal Care Committee (protocol #1001B-11).

**Museum Sample Collection**

Historic whip-poor-will tissue samples were obtained from the collections of the Royal Ontario Museum (Toronto, Canada) and the Canadian Museum of Nature (Ottawa, Canada). We collected samples from a total of 63 whip-poor-wills obtaining adult feathers from 26 individuals (1 unknown sex), juvenile feathers from 25 individuals (4 unknown sex) and claws from 37 individuals. We stratified this sampling as evenly as possible across geographic regions in Ontario, decades (from 1880 to 2006), age classes, and between males and females. We preferentially selected specimens collected between 15 May and 15 August, so that the collection location was more likely to represent the breeding/molt location. Some birds (N = 12) collected during migration periods were also included, but because Ontario is the northern limit of their breeding range the breeding or natal sites of these migrants is also likely to have been within our sampling area.

To test for an underlying shift in historic baseline isotope values, we obtained tissue samples from three insect species likely to represent components of the whip-poor-will diet: Biston betularia, a widespread herbivorous moth of a size likely to be eaten by whip-poor-wills; Phyllophaga anxia, a herbivorous beetle of a genus known to be consumed by whip-poor-wills (personal observation); and Colymbetes sculptilis a small species of predacious diving beetle, a group which disperses nocturnally and therefore could be a source of aquatic derived food for nocturnal aerial insectivores. These species are all widespread in the same regions and habitats as whip-poor-wills and had the best temporal coverage in museum collections. We sampled specimens of each species from most decades between 1880 and 2013 that were collected across the same general regions of Ontario as the birds (with the addition of a few samples from neighboring regions of southern Quebec; Figure 1). Insect tissues were obtained from the Canadian National Collection of Insects (Agriculture and Agri-Food, Ottawa, Canada) and the Royal Ontario Museum. We sampled a single hind leg from each specimen (47 B. betularia; 29 P. anxia; 24 C. sculptilis).

**Stable Isotope Analysis**

We cleaned samples by rinsing with deionized water (Wolf et al., 2011, 2013) and air or oven dried for 48 h. We did not use solvents because there was a risk of losing the tiny claw fragments during more complex cleaning processes, but we acknowledge that this may increase variance due to lipid contamination (Paritte and Kelly, 2009; Valladares et al., 2010). We sampled and minced ~50 μg of the feather barbs, avoiding the rachis. Claw and insect fragments were ground and analyzed whole. All samples were placed in tin capsules. We did not perform any lipid extraction because both feathers and claws have sufficiently low lipid content (confirmed by C:N ratios < 4; Post et al., 2007). We were also primarily interested in temporal change in the δ¹³N values of their insect prey, and this tends to be less dependent on lipid content (Logan et al., 2008; Kaufman et al., 2014). The University of Waterloo Environmental Isotope Laboratory analyzed all samples using combustion conversion of sample material to gas through an 1108 Elemental Analyzer (Fisons Instruments) coupled to a Delta Plus (Thermo) continuous flow isotope ratio mass spectrometer (CFIRMS). Stable isotope ratios are expressed with the δ notation (parts per thousand—‰) relative to atmospheric N₂ and Vienna PeeDee Belemnite for δ¹⁵N and δ¹³C values, respectively. Carbon isotope values were corrected for atmospheric change in δ¹³C, known as the Suess effect, based on a formula (#4) from Verburg (2007), and reported relative to year 2000 levels for ease of comparison with other ecological studies. Data quality was monitored and corrections made using
TABLE 1 | Contemporary tissue sample sizes and mean ± standard deviations of isotope values from A. vociferus breeding at three localities in Ontario (see Figure 1).

<table>
<thead>
<tr>
<th>Tissue growth location</th>
<th>Feathers</th>
<th>Rainy river</th>
<th>Norfolk</th>
<th>Frontenac</th>
<th>Claws</th>
<th>Wintering grounds</th>
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</thead>
<tbody>
<tr>
<td><strong>INDIVIDUALS SAMPLED</strong></td>
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<tr>
<td>Adult</td>
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<tr>
<td>Male</td>
<td>13</td>
<td>7</td>
<td>16</td>
<td></td>
<td>56</td>
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<tr>
<td>Female</td>
<td>6</td>
<td>0</td>
<td>9</td>
<td></td>
<td>20</td>
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<tr>
<td>First-year Male</td>
<td>2</td>
<td>5</td>
<td>4</td>
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<td>14</td>
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<tr>
<td>First-year Female</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td></td>
<td>10</td>
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<td><strong>δ¹⁵N-VALUES</strong></td>
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<td>Adult</td>
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<tr>
<td>Male</td>
<td>7.4 ± 0.8A</td>
<td>6.7 ± 0.7A</td>
<td>6.9 ± 0.5A</td>
<td>6.2 ± 0.7A</td>
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<tr>
<td>Female</td>
<td>6.9 ± 1.4</td>
<td></td>
<td>6.9 ± 0.4</td>
<td>6.4 ± 1.0A</td>
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<tr>
<td>First-year Male</td>
<td>5.6 ± 1.8</td>
<td>4.7 ± 0.7</td>
<td>5.0 ± 0.6</td>
<td>6.6 ± 1.1A</td>
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<tr>
<td>First-year Female</td>
<td>3.1</td>
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<td>5.7 ± 0.2</td>
<td>7.4 ± 1.2A</td>
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<td><strong>δ¹³C-VALUES</strong></td>
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<td>Adult</td>
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<tr>
<td>Male</td>
<td>−24.4 ± 0.2A</td>
<td>−24.8 ± 0.4AB</td>
<td>−24.9 ± 0.7B</td>
<td>−23.0 ± 0.6A</td>
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<tr>
<td>Female</td>
<td>−24.1 ± 0.3</td>
<td></td>
<td>−24.6 ± 0.5</td>
<td>−22.6 ± 0.9A</td>
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<tr>
<td>First-year Male</td>
<td>−25.3 ± 0.1</td>
<td>−24.6 ± 0.7</td>
<td>−25.1 ± 0.2</td>
<td>−21.8 ± 1.1B</td>
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<tr>
<td>First-year Female</td>
<td>−24.5</td>
<td></td>
<td>−25.1 ± 0.2</td>
<td>−22.1 ± 0.9B</td>
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</table>

Superscripts indicate significant differences in isotope values between breeding localities for adult males and between age (first-year refers to juvenile feathers and first-winter claws sampled from 1-year-old birds) and sex classes for all samples assessed using mixed-models that control for sampling location.

Statistical Analysis
To understand factors underlying individual variation in isotope signatures within contemporary bird tissues, we first examined how δ¹⁵N values varied with age, sex, and δ¹³C, using linear mixed-effect models with sampling location included as a random effect. Both the age and sex may influence metabolism and nutritional states of individuals, which can influence fractionation of nitrogen (Robbins et al., 2005; Caut et al., 2008; Cherel et al., 2014), and δ¹⁵N and δ¹³C values are known to co-vary (Kelly, 2000). Inclusion of sampling location as a random effect allowed us to estimate average coefficient values from which an certified international reference materials (ammonium sulfate: IAEA-N1 = +0.43‰ and N2 = +20.41‰ δ¹⁵N, cellulose: IAEA-CH3 = −24.72‰ δ¹³C, sucrose: IAEA-CH6 = −10.45‰ δ¹³C, L-glutamic acid: USGS-40 = −4.52‰ δ¹⁵N/−26.39‰ δ¹³C) and in-house standards (bovine liver: NIST 1577b = +7.72‰ δ¹⁵N/−21.60‰ δ¹³C, cellulose: EIL-72 = −25.5‰ δ¹³C, ammonium sulfate: EGC-3 = +0.77‰ and JSEC-01 = +20.20‰ δ¹⁵N) that were calibrated using the same reference materials. No < 20% of every sample run were standards and reference materials. Laboratory measurement error for these standards were all < ±0.3‰ for nitrogen and ±0.2‰ for carbon. Measurements from the same feather (N = 21), claw (N = 16), and insect leg (N = 12) were repeatable to 0.26 ± 0.15‰, 0.33 ± 0.22‰, and 0.26 ± 0.15‰ respectively for nitrogen and 0.14 ± 0.13‰, 0.39 ± 0.31‰, and 0.20 ± 0.15‰ for carbon. Claw samples were often too small for within sample repeatability estimates so we sampled both feet for 15 birds and found the repeatability between feet to be 0.59 ± 0.50‰ for nitrogen and 0.51 ± 0.48‰ for carbon.
we could make unconditional inferences about other locations. We also used analysis of variance and Tukey HSD tests to determine if feather $\delta^{15}N$ and $\delta^{13}C$ values differed between the three breeding sites in Ontario for adult males.

To model change in the $\delta^{15}N$ values of bird tissues over the past 130 years, we fit models that included tissue growth year, bird age, and $\delta^{13}C$ value of the sample as fixed effects, thereby controlling for all the covariates found to influence $\delta^{15}N$ values within the contemporary samples (see section Results). We fit separate models for claw tissue grown on the non-breeding grounds and feathers grown in the breeding season. Food deprivation could be more pronounced in younger faster-growing birds, so we tested if an interaction between bird age and year improved model fit using a likelihood ratio test (LRT). Baseline isotope ratios of nitrogen and carbon can co-vary (Kelly, 2000), so we report coefficients for models both with and without the $\delta^{13}C$ value as a covariate to determine if the magnitude of the Suess effect or factors underlying this co-variation could influence our conclusions.

To determine if baseline $\delta^{15}N$ of prey on the breeding grounds has changed over the last 130 years, we used a mixed-effect model with collection year as a fixed effect and species as a random effect. Using species as a random effect allows unconditional inferences about other species in the insect community. Without lipid extraction, $\delta^{13}C$ values of insects are not reliable when C:N $> 4$ (Post et al., 2007), so we do not control for $\delta^{13}C$ values in the model we report. We did, however, test the effect of controlling for these potentially lipid-biased $\delta^{13}C$ values, and doing this does not significantly or qualitatively change our results (see Supplemental Table 2). We test the power of our analysis to detect a slope of the same magnitude using the R package “simr” (Green and MacLeod, 2016), which conducts a power analysis of generalized linear mixed models by simulation.

Finally, because changes in $\delta^{13}C$ values (due to shifts in habitat or agricultural practices) could mask changes in trophic level, we tested whether there was any evidence of temporal variation in $\delta^{13}C$ within the museum tissues. For each type of whip-poor-will tissue, we fit a model that included growth year, age, and sex as fixed effects. For insects, we used a single linear mixed-effect model with collection year and the ratio of C:N as fixed effects, and species included as a random effect. Because the ratio of C:N can be correlated with lipid content, this ratio can be used to control for variation in lipid content between samples (Post et al., 2007; Logan et al., 2008).

For each tissue type, we checked for non-linearity in the temporal change in $\delta^{15}N$ and $\delta^{13}C$ values of museum tissues using a generalized additive model (GAM; GAMM for the mixed effects) using an identity link, a cubic regression spline, and cross-validation to choose the optimal amount of smoothing in the R package “mgcv” (Wood and Wood, 2016). In all cases the best model indicated that temporal change was linear, so we used linear models in subsequent analyses. We then incorporated contemporary tissue samples into these linear models before calculating parameter estimates. To account for a much larger number of samples from contemporary bird tissues than from museum collections, we bootstrapped 1,000 samples of 10 from our contemporary samples and refit the model to each sample.

Combined, the models provide a distribution of parameter estimates, for which we report a mean and the range that encompasses 95% of the estimates.

We conducted all analyses in R version 3.2.1 (R Development Core Team, 2015). We fit all linear models using either the “glm” (for linear) and “lme” (for linear mixed effects) functions in the R package “nlme” (Pinheiro et al., 2016). For mixed-effect models, we compared models with random slopes and intercepts, vs. random intercepts only using LRTs. Models were fit using maximum likelihood (ML) for comparisons between of models with different fixed effects, but we report parameter estimates and 95% confidence intervals from models fit using restricted maximum likelihood (REML) to reduce the potential for bias due to small sample sizes (Zuur et al., 2009). To facilitate parameter estimation and comparisons between continuous and categorical variables, we centered year variables to 1950, and centered and scaled carbon values by two standard deviations (Schielzeth, 2010). Finally, we inspected the residuals of all models for normality and homogeneity of variance. We found no evidence of spatial autocorrelation in our analyses: residuals were not spatially clustered and there was no pattern in the semivariance with distance between sampling locations (feathers: $r = −0.023, p = 0.92$; insects: $r = −0.39, p = 0.09$). We calculated both marginal and conditional $R^2$ for all mixed-effect models (Nakagawa and Schielzeth, 2013) and used Kenward-Roger approximation to estimate $p$-values (Arnau et al., 2014).

RESULTS

In contemporary bird tissues, we found evidence that $\delta^{15}N$ varied with $\delta^{13}C$ values and the age, but not the sex, of the bird (Figure 2; Supplemental Table 1). The $\delta^{15}N$ values of winter-grown claws increased as $\delta^{13}C$ values increased, with a 2 SD increase in $\delta^{13}C$ value of claw tissue predicted to increase $\delta^{15}N$ values by 0.9‰ (95% CI: 0.6–1.2; Figure 2B). In contrast, $\delta^{15}N$ values of feathers grown on the breeding grounds decreased slightly as $\delta^{13}C$ value increased, with 2 SD increase in $\delta^{13}C$ value predicted to decrease $\delta^{15}N$ values by 0.5‰ (95% CI: −0.9 to 0.0). An effect of age on $\delta^{15}N$ value was only detected in the feathers. While controlling for variation in $\delta^{13}C$ values, $\delta^{15}N$ values of nesting-grown feathers were −1.9‰ (95% CI: −2.4 to −1.5) lower than that of feathers from adults (Figure 2A). By the time young birds reached the end of their first winter this difference disappeared, and the $\delta^{15}N$ values of claw tissue overlapped with that of older birds (0.4‰o, 95% CI: −0.0 to 0.8; Figure 2B). Sexes did not appear to differ in $\delta^{15}N$ value of tissues grown during either season (claw: 0.0‰o, 95% CI: −0.4 to 0.3; feather: 0.0‰o, 95% CI: −0.4 to 0.5). There was no evidence that co-variation between the two isotopes differed between feathers grown at different breeding locations (probability that slopes differed with locality LRT $\chi^2_2 = 0.12, p = 0.94$) and the only significant difference in average values of adult male feathers was in the $\delta^{13}C$ values of feathers grown in Frontenac County when compared with those grown in Rainy River District [feather $\delta^{13}C$: $F_{(2, 33)} = 4.27, p = 0.02$; feather $\delta^{15}N$: $F_{(2, 33)} = 3.04, p = 0.06$; Table 1].
Whip-poor-will claw and feather δ¹⁵N values declined by 2.0 and 2.4‰, respectively over the past 130 years. For winter-grown claws, δ¹⁵N values declined by 0.015‰ per year (95% range: −0.011 to −0.018; Figure 3A). For breeding ground-grown feathers, the δ¹⁵N values declined by −0.019‰ per year (95% range: −0.016 to −0.022; Figure 3B). Our estimate of change in δ¹⁵N value per year on the breeding grounds is slightly larger when δ¹³C value is not included as a covariate in the model (−0.020‰ per year, 95% range: −0.017 to −0.023), while not controlling for changes in δ¹³C value has the opposite effect on wintering δ¹⁵N values (Supplementary Table 2). Juvenile feathers share a similar rate of change with time (df = 1, LRT = 1.75, p = 0.19), but δ¹⁵N values are offset by −1.4‰ (95% range: −1.3 to −1.6) from those of adults.

In contrast, there was no evidence of depletion in δ¹⁵N values of insect tissues over time (−0.002‰, 95% CI: −0.019 to 0.015; Figures 3C–E). We also found no evidence that temporal change varied among species (LRT x² = 2.77, p = 0.25). However, individuals had highly variable δ¹⁵N values within each species. Therefore, despite collecting a relatively large number of insect samples (N = 100), our power to detect a slope of the same magnitude observed in the whip-poor-will samples was relatively low (62% of 5,000 simulations). The confidence intervals for insects are so broad that they range from a positive slope of 0.015‰ to a negative slope that overlaps with the predicted range we found for bird feathers.

We found some indication of a long-term change in δ¹³C values of winter-grown bird tissues, which could be associated with changes in habitats or agricultural practices (Figure 4). The δ¹³C values of claws grown on the wintering grounds increased by 0.010‰ per year (95% range: 0.006–0.013). In contrast, δ¹³C values of feathers grown on the breeding grounds did not change significantly (0.003‰ per year, 95% range: −0.001 to 0.004). Similarly, there was little evidence of change in δ¹³C values of insect tissues (0.007‰, 95% CI: −0.003 to 0.018). This lack of change through time in δ¹³C values of insect tissues was consistent whether or not a significant relationship between C:N ratio and δ¹³C values (−0.43‰, 95% CI: −0.79 to −0.07; Supplemental Figure 2) was controlled for in the model.

**DISCUSSION**

Widespread population declines in aerial insectivores occupying different habitats suggest that insect availability could be the common factor limiting their populations. If insect population declines are limiting food availability or quality for these species, higher trophic level species are likely to disappear from their diet first. Given the absence of long-term data on the abundance of insects, stable isotope ratios of museum specimen tissues provide an indirect record of diet and habitat change through time. Using museum tissues, we found a decline of 1.4–2.8‰ in δ¹⁵N values in insect tissues over the past 130 years. This result, coupled with the comparative stability of δ¹³N values in insect tissues, is consistent with contemporary whip-poor-will populations feeding lower in the food web than in the past. This pattern was observed in both adult and juveniles, and was slightly stronger on the breeding grounds than on the wintering grounds. While these results all support the possibility that these population declines are due to changes in abundance of high quality, higher trophic-level prey, we caution that stable isotope studies of terrestrial food chains require additional tests of baseline change and do not exclude other possible drivers of population decline.

Temporal change in δ¹⁵N values of animal tissues can arise through a change in the isotope ratio of the nitrogen sources or types of primary producers supporting the prey consumed, or through a change in trophic-level of the species being sampled. While our analysis attempts to tease apart these alternative hypotheses, due to exceptionally high variance in the δ¹⁵N values of insect tissues, we cannot reject any hypothesis conclusively. We did not find evidence of a significant change in δ¹⁵N values of insect tissues that could indicate a shift in the “baseline” isotopes available to primary producers on a regional scale, but due to the high within-species variance in δ¹⁵N values, the confidence interval on this trend overlaps with our estimate of change in birds. This increasing power to detect long-term changes in baselines at higher trophic levels was also found for atmospheric changes in δ¹³C (Bump et al., 2007). Evidence that foliar δ¹⁵N values decline by as much as 2‰ along a successional

**FIGURE 2** | The population level predicted effect on δ¹⁵N of a change in δ¹³C of bird tissues from a mixed-effect model controlling for breeding site: (A) feathers grown by adults on the breeding grounds (green squares) or by nestlings being fed by parents (orange diamonds), and (B) winter-grown claw from birds during their first year of life (turquoise diamonds) and older adults (blue circles). Filled symbols are males, open are females.
FIGURE 3 | Measured changes in δ^{15}N-values of bird and insect tissues over time: (A) winter-grown A. vociferus claws, (B) A. vociferus feathers grown by adults on the breeding grounds (squares) or juveniles still being fed by parents (diamonds), (C) Biston betularia, (D) Phyllophaga anxia, and (E) Colymbetes sculptilis. The lines show the mean predicted relationship from linear models controlling for δ^{13}C based on all historic samples and 1,000 bootstrapped sub-samples of contemporary bird tissues. The gray shows the variation in predicted slopes. Bird illustrations are courtesy of Marinde Out.

gradient with up to a century of time since disturbance (Compton et al., 2007; Wang et al., 2007) suggests that afforestation of breeding habitats in the larger study region may produce a baseline shift that would result in the pattern we observed in bird feathers (Foster, 2000; Cadman et al., 2007). In contrast, ongoing deforestation on the wintering grounds makes this mechanism seem less likely to explain the similar rate of depletion found in claws (Wassenaar et al., 2007; FAO, 2011; Aide et al., 2013); however, data regarding wintering habitat use in Central America will be required (English et al., 2017a). Furthermore, although complicated by complex local variation (Bump et al., 2007), tree-ring cores sampled in our study region also fail to show a consistent decline in δ^{15}N over the past 130 years. Instead, tree-rings provide evidence that local deforestation can cause relatively short-term (~20 years) increases in δ^{15}N values (Bukata and Kyser, 2005) and long-term (>100 years) declines of ≤1‰ in relatively undisturbed forest (Bukata and Kyser, 2007). Likewise, anthropogenic nitrogen deposition has been implicated in a decline in the δ^{15}N values of bone collagen in Japanese Sika Deer (Cervus nippon), but the magnitude of this change was only 0.4‰ (Matsubayashi et al., 2015). Given our minimum estimate of change in δ^{15}N values of bird tissues is close to the maximum reported for any of these mechanisms, and that none of the insect species sampled showed any similar trend, it appears unlikely that these mechanisms alone have caused this magnitude of change on average across the primary production consumed by all insect prey.

Alternatively, while the baseline δ^{15}N value of the habitat occupied by the insect species we chose to sample may have remained the same, habitats supporting other phytophagous
prey species may have changed. Although whip-poor-will forage relative close to the ground, the volant nature of their prey makes it likely that some prey may move in from surrounding habitats that might not appear to be used by whip-poor-wills. In particular, changes in forest cover and agriculture may have shifted ratios of C3 and C4 plants, moisture regimes, and fertilizer use, all of which could influence baseline $\delta^{13}C$ or $\delta^{15}N$ values (White et al., 2012; Augusto et al., 2015; Popa-Lisseanu et al., 2015). C4 plants (many grasses and crops like corn, sorghum, and sugar cane) have higher $\delta^{13}C$ than C3 plants and tend to occur in more disturbed, and therefore more $^{15}N$ enriched habitats (Kelly, 2000). Therefore, the positive relationship between $\delta^{15}N$ and $\delta^{13}C$ values across all winter-grown claw tissues (Figure 2B) could be a result of higher proportions of C4 plants growing in the driest, most disturbed habitats that are supporting phytophagous insect prey. Irrigation of C3 plants grown on more disturbed sites, more high trophic level prey originating from wetter habitats, or the addition of low $\delta^{15}N$ synthetic fertilizers to C4 crops could dilute, or even reverse, this relationship. Indeed, a weak negative relationship between $\delta^{15}N$ and $\delta^{13}C$ values of feathers grown on the breeding grounds (Figure 2A) could be related to differences in the amounts of fertilizer applied due to the economic differences between these regions. Regardless of the mechanism, by including the $\delta^{13}C$ value of each sample as a covariate in our models of $\delta^{15}N$ change in bird tissues, we attempt to control for some of the potential changes in the habitats that support prey insects. As a result, our estimate of change in $\delta^{15}N$ values of tissues grown during the breeding season is made more conservative, but does not alter our conclusion that the mean $\delta^{15}N$ value of birds has declined over time.

The use of stable isotopes to illuminate trophic ecology of species is a powerful tool, but is complicated by differences in fractionation rate (i.e., discrimination factors) between species, individuals of different nutritional and physiological states, and between tissues within individuals (Robbins et al., 2005; Caut et al., 2008; Cherel et al., 2014). By looking at patterns within tissues of a single species, we can avoid most of these issues. The remaining variance in nutritional and physiological state are both potentially related to the diet we are attempting to assess. The lower $\delta^{15}N$ values of juvenile feathers, which are all grown concurrently during the first weeks of life, is likely at least partially the result of reduced discrimination (that can be up to 0.95%) due to very little nitrogen being wasted under conditions of rapid growth and/or food restriction (Sears et al., 2008; Hertz et al., 2015). Because discrimination factors vary between species and with diet quality (Robbins et al., 2005), it is possible that the offset of 1.4% that we observe is still entirely due to extremely rapid growth rates. Alternatively, some of the difference may be due to adults preferentially provisioning juveniles with lower trophic-level lepidopteran prey (Cink et al., 2017). By focusing our sampling on tissues grown at relatively predictable rates and during specific seasons, differences in diet between individuals become more meaningful. Feathers grown concurrently by juveniles and the adults provisioning them represent diet during the period most crucial for determining population growth rates. Lower $\delta^{15}N$ values over time in both adult and juvenile feathers could represent either increasing nutritional stress, a shift in the baseline $\delta^{15}N$ values of prey types consumed, or a reduction in the trophic level of prey consumed. Assessing temporal changes in the offset between adults and juveniles could help distinguish between these alternatives, however a larger number of tissue samples from the early 1900’s would be needed to ensure the statistical power to detect differences in slope.

Long-term monitoring studies are rare, expensive, and most conservation questions are rarely anticipated in time to establish appropriate protocols. Stable isotope studies provide a unique opportunity to harness the ecological history contained in museum collections (Darimont and Reimchen, 2002). However, care must be taken to adequately account for differences in discrimination between tissues and species, seasonal and geographic variation in isotopic baselines, and the physiological state of individual animals. While this opportunity has been explored quite extensively for marine ecosystems, its application to complex terrestrial food webs remains relatively untested. This added complexity is highlighted by our finding of extremely high variability (1.5–13.0‰) in the $\delta^{15}N$ values of individuals within a single insect species, B. betularia. This high variability is likely due to this species being relatively generalist in its habitat and host plant choices and each individual insect likely having acquired its dietary nitrogen from a single plant, each of which grew under variable local conditions including moisture regimes and nitrogen sources. As trophic-level and the geographic area over which an organism acquires its nutrition increases, the variance between individuals should decrease (McNabb et al., 2001; Bump et al., 2007; Gibb and Cunningham, 2011). The result of this high variability was that even with 100 insect samples, we only had a 62% chance of finding a significant trend of the same magnitude that we found in the bird feathers. With this in mind, we suggest that rather than attempting to

![Figure 4](https://example.com/figure4.jpg)
sample lower trophic levels by sampling prey species directly, future studies should use a comparative approach and sample species with different diets, but similar scales of movement and geographic distribution of their food sources (Thompson et al., 1995). Alternatively, as compound-specific stable-isotope analyses become more affordable, it will be possible to determine baseline values and thereby more accurately assess trophic-level changes from within a single tissue sample (McMahon et al., 2015; Hebert et al., 2016; Ostrom et al., 2017).

Despite these challenges, the ability to decode the historical record of ecological change locked inside museum collections has tremendous potential to inform our understanding of ecological change and aid in conservation decisions. For aerial insectivores, a significant change in dietary isotopes of whip-poor-wills over the past 130 years adds to the mounting evidence that population declines for many of these species may be related to changes in food supply. Insect abundance has previously been shown to predict both the presence and abundance of whip-poor-wills and bats (Wickramasinghe et al., 2004; English et al., 2017b) and changes in insect prey species composition appear correlated with population declines in two species of swift (Nocera et al., 2012; Pomfret et al., 2014). We believe that analysis of temporal trends in stable isotope ratios of other aerial insectivores, and further exploration of potential shifts in terrestrial baselines, will be crucial to assessing the relative importance of diet to the population trajectories of this guild. If population trajectories are indeed linked to changes in diet for most of these species, understanding this will be an important first step toward making effective, data-driven conservation decisions.

**DATA AVAILABILITY**

Isotope data is included in Supplementary Data Sheet 1.

**AUTHOR CONTRIBUTIONS**

PE: conceived the idea; PE, DG, and JN: designed methodology; PE and JN: coordinated data collection; PE: analyzed the data and led the writing of the manuscript; All authors contributed critically to the drafts and gave final approval for publication.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2018.00014/full#supplementary-material

**REFERENCES**


English et al. Long-Term Change in Insectivore Isotopes


**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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