Stable isotope analysis of local avian diets

August 2008

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ABSTRACT

Stable Isotope Analysis (SIA) quantifies the proportion of ^{13}C and ^{15}N within an organism's tissue and may be used in trophic and nutrient assimilation analysis (Hobson and Sealy 1991). Birds were mist-netted at the North Maple River MAPS site between July 17 and August 1, 2008. Birds were identified and banded according to MAPS protocol and blood samples of 100 µl were taken. Food items were also collected at site and sorted into three groups: berries, aquatic insects, and terrestrial insects. Blood and diet samples were dried and analyzed for $\delta^{13}C$ and $\delta^{15}N$. Bird isotope signatures were adjusted with discrimination factors and plotted within a mixing model. Proportions for species within model were calculated using IsoSource (interval=1.0, tolerance=0.05). All species, excluding Yellow Warblers, Black-capped Chickadees, and Gray Catbirds, assimilated at least 50% of nutrients from the terrestrial food group. SIA is a new tool for diet analysis and more research is necessary to determine more accurate discrimination factors for North American avifauna.

INTRODUCTION

Stable Isotope Analysis (SIA) is a rising trend in the study of nutrient and trophic level distributions of an ecosystem. SIA quantifies the proportion of stable isotopes ^{13}C and ^{15}N in the tissue of an organism. It is possible to determine the dietary origin of nutrients incorporated into the consumer's tissue because these isotope ratios can be correlated to those of the diet (Hobson and Sealy 1991). While conventional diet-assay methods provide information about what was consumed via food matter (in the form of pellet, feces or alimentary tract contents), they do not indicate what nutrients are incorporated into tissues. SIA quantifies the assimilation of organic material into the tissues of organisms and also the proportions of their contribution to tissue growth (Herrera M. *et al*. 2006, Inger and Bearhop 2008). Analysis of the representative isotope ratios in avian tissues can show the predominant source of nutrition in each specimen, especially when two or more food options with distinct isotope ratios are available (Hobson and Clark 1992*a*, Herrera M. *et al.* 2006). Identifying one bird species' "isotopic niche" may be a significant contribution to an understanding of its foraging behavior, ecological niche and trophic level (Inger and Bearhop 2008).

Different tissues within an organism assimilate nutritional matter at different rates. The stable isotope ratio of a specific tissue reflects the ratios of the dietary elements the animal consumed at the time of the tissue's growth (Inger and Bearhop 2008). Isotope assimilation rates correlate with the longevity of the tissue examined. Considering its relatively quick turnover rate and valuable quality as a non-destructive material for SIA, whole blood is a useful source of time-based information about a bird's diet (Hobson and Clark 1992*a*, 1993). In addition to determining the assimilation rate of stable isotopes into the SIA source tissue, the magnitude of isotope fractionation, or discrimination, must also be considered when using stable isotopes in an

avian diet study (Hobson and Clark 1992*b*, Inger and Bearhop 2008). In ecological studies, the discrimination factor of a species describes the degree to which heavier isotopes are selected against in biological reactions, resulting in an accumulation of the isotope in the tissues of the organism (Szepanski *et al.* 1999). Limited isotope enrichment must be corrected when comparing prey and consumer isotope signals (Phillips and Gregg 2001).

Our study uses whole blood stable-carbon and stable-nitrogen isotope analysis of avifauna of mixed-hardwood forests on the Maple River in Northern Lower Michigan to investigate their nutrient assimilation at their summer breeding grounds. An understanding of the dietary history of avian species has been limited to stomach contents examined during carcass dissection (Cornell Lab of Ornithology website). To date, few studies have employed SIA in creating an accurate description of the dietary profile of wild birds in an ecosystem. We have quantitatively shown that the stable-carbon ($^{13}C/^{12}C$) and stable-nitrogen ($^{15}N/^{14}N$) of whole blood samples from a diverse group of species from a similar habitat resembled the ratios in potential food items collected at the same site.

MATERIALS AND METHODS

Sampling

Data was collected at the Maple River North MAPS (Monitoring Avian Population and Survivorship) banding site located outside Pellston, Emmett Co., Michigan on Douglas Lake Road. Data was collected for 6 days between July 17 and August 1, 2008 from 6 to 11 a.m.

Birds were collected using 10 mist nets (12 m x 3 m) at the site. Mist nets were monitored every 25 minutes to collect specimens and to minimize the stress of netted birds. Once caught, the birds were placed in bird bags for transport to a central processing station.

Birds were weighed, sexed, aged, banded and identified by species according to MAPS protocol. The band numbers prevented birds from being sampled more than once. Whole blood was chosen as a non-destructive isotope source material. Blood was taken from the brachial artery. The feathers covering the artery were removed and the area was sterilized with isopropyl ethanol to prevent infection. The artery was punctured using a sterile needle and the pooling blood was taken up using a capillary pipet. The blood was placed in 1.5 ml centrifuge vials containing 70% ethanol to preserve the blood until drying. Styptic powder was used to aid in clotting before release of the bird. Enough blood to produce 2 µg dry-weight was necessary to run heavy nitrogen SIA; this translates roughly into 100 µl of whole blood.

Insects and berries were collected at the site on two separate occasions. Leaf litter samples processed in Berlese funnels for 24 hours to extract insects. Insects at the site were sampled using sweep and aerial insect nets and a light trap after dark. All food specimens were stored in ethanol until processing. To prevent contamination, samples collected at the site were handled using tweezers.

Stable Isotope Analysis

For SIA, insects were sorted by location collected and further separated by order; berries were sorted by genus (Table 1). Each blood and food item sample was labeled, and dried at 50°C for 24 hours. After 24 hours samples were pulverized and dried for another 24 hours at 50°C. 2 µg of each dried and ground sample were placed in the mass spectrometer (Thermo Finnigan Delta+ XP, Thermo Electron co.). The mass spectrometer was coupled with an elemental analyzer (Costech 4010, Costech Analytical Technologies, Inc.) to separate the isotopes of carbon and nitrogen. Isotope ratios were determined by the following equation:

$$\delta = ((R_{sample}/R_{standard})-1) \times 1000$$

where δ represents the isotope ratio, R_{sample} is the fraction of heavy to light isotopes, and $R_{standard}$ is the international standard isotope ratio based on carbon in fossil limestone and atmospheric nitrogen (Herrera M. *et al*, 2006).

Statistical Analysis

Orders of food groups were plotted according to carbon and nitrogen δ values (Table 2). Four food groups were originally chosen to average as diet sources tested in our study (Table 1). Of the four food group averages we sampled, significant difference in $\delta^{15}N$ isotope ratios was determined using an independent t-test between berries and aquatic specimens ($p<0.01$), berries and leaf litter specimens ($p<0.05$), berries and understory specimens ($p<0.01$), aquatic specimens and leaf litter specimens ($p<0.01$) and aquatic specimens and understory specimens ($p<0.01$). No significant difference was determined between leaf litter and understory specimens. These two groups were averaged together into terrestrial insects and significant difference was shown between terrestrial specimens and berries and aquatic specimens ($p<0.01$ and $p<0.001$, respectively; Figure 1).

$\delta^{13}C$ and $\delta^{15}N$ ratios were corrected by discrimination factors of 2.0‰ and 2.5‰ respectively. Discrimination factors were chosen based on reported averages of multiple studies on whole blood fractionation in avian species (Herrera M. *et al*, 2006). $\Delta^{13}C$ and $\Delta^{15}N$ were averaged and then plotted together with isotope ratios of aquatic, berries and terrestrial insects (Figure 1). An isotope mixing model, Isosource (EPA 2007), was used to determine possible proportions of the three food groups' isotopic contribution to each species' whole blood isotope mixture (Phillips and Gregg 2003).

RESULTS

A total of 45 birds were caught representing 14 species (Table 3). 6 species (American Redstart, American Robin, Common Yellowthroat, Hairy Woodpecker, Northern Waterthrush, and Yellow-bellied Sapsucker) fell outside the diet triangle and the possible proportions of the three diet items were not calculated. Ranges of diet composition were calculated for eight species within mixing model triangle (Black-capped Chickadee, Black-and-white Warbler, Gray Catbird, Hermit Thrush, Ovenbird, Rose-breasted Grosbeak, White-throated Sparrow, and Yellow Warbler) using a tolerance level of 0.05 and in increments of 1.0. Ranges for possible diet compositions of the eight species within the diet triangle varied only slightly (between 0-0.03). The majority of birds within the triangle, excluding Hermit Thrushes, had diets composed of all three diet items (Table 4). Black-and-white Warblers, Ovenbirds, the Rose-breasted Grosbeak, and the White-throated Sparrow had diets composed of at least 50% terrestrial insects and the Yellow Warbler assimilated a majority of aquatic insects (60-61%). Black-capped Chickadees and Gray Catbirds had diets composed of 33-34% berries, 20-21% aquatic insects, 45-47% terrestrial insects, and 7-9% berries, 41-43% aquatic insects, 48-51% terrestrial insects, respectively.

DISCUSSION

This study created a model for the nutrient assimilation patterns of avian populations on the Maple River in Michigan. Our findings show that nearly every species' isotopic ratio we sampled had the greatest proportion of isotopic contribution from the terrestrial group. Some species had greater assimilation proportions from either the aquatic or berries group. Birds may

eat many different types of insects but isotopic analysis highlights which food sources are being assimilated; no species had a dedicated contribution from only one isotope source.

The chosen grouping system developed reflects three statistically different trophic levels, because $\delta^{15}N$ values tend to reflect the trophic level of an organism (Inger and Bearhop 2008). The terrestrial group consisted of food item samples that had a large range in both the $\delta^{15}N$ and $\delta^{13}C$ ratios. This could explain why several sampled species were excluded from our mixing model. Calculated proportions of the food group contributions were also affected by this variance in the Terrestrial food group average ratio. The fact that several species fell outside of our mixing triangle may also indicate that another food source may not have been accounted for. More extensive sampling of food items in the experimental habitat may result in a more accurate representation of nutrient assimilation contributions in avian species.

In ecological stable isotope studies of foraging patterns, discrimination of metabolic processes against heavier isotopes accounts for an amplification of $\delta^{15}N$ and $\delta^{13}C$ ratios in consumer tissues. The discrimination factors used in this study are averages of several factors that have been reported for a wide variety of avian species and habitats (Pearson *et al.* 2003, Hobson and Bairlein 2003, Herrera M. and Reyna E. 2007, Carleton and Martinez del Rio 2005). Because actual discrimination factors of species in mixed-hardwood forest are unknown, the application of nonspecific corrections could have placed avian specimen ratio averages incorrectly relative to this mixing model. Long term SIA of diverse bird families and species of North America would provide greater insight into discrimination factor values.

Whole blood is an appropriate source material for SIA because of its minimized impact on avian samples and short turnover value of tissue generation (Hobson and Clark 1992*a*). The ratios detected in whole blood samples represent average nutrient assimilation in the two weeks

prior to sample collection. This experiment reports dietary patterns of a limited time period, while nutritional isotope blood ratio changes seasonally with the changing abundance of resources (Herrera M. *et al* 2006). Yearlong studies involving non-migratory species at this sampling site could give more insight into seasonal dietary shifts.

The use of SIA in reinforcing what is often anecdotally inferred about avian dietary biology is very promising. The number of studies quantifying metabolic assimilation rates in avian species is steadily increasing. This information creates a foundation for SIA application in modeling trophic relationships within ecosystems, although studies that incorporate a variety of avian species and their nutrition sources within an ecosystem have only recently begun to be published.

ACKNOWLEDGEMENTS

We would like to thank Jennifer Mills, Steven Mateyka, Luke Rosier, Kim Greene, Ted Anderson, Stephanie Schubel, and Tom Dietsch for helping us mist net. We would also like to especially thank Tom for his guidance throughout the research process. And finally we thank Brian Scholtens for his advice on collecting insects and Mike Grant for his assistance with our specimen analysis.

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TABLES

Food Group	
	Order
Aquatic	Odonata
	Trichoptera
	Ephemeroptera
Berries	**Genus**
	Lonicera
	Prunus
	Amelanchier
	Viburnum
Terrestrial	**Order**
leaf litter	Coleoptera
	Hymenoptera
	Diplopoda
	Annelida
understory	Lepidoptera
	Arachnids
	Coleoptera
	Orthoptera
	Chrysopidae
	Homoptera

Table 1. Grouping of collected food items in experimental habitat. Terrestrial group is an average of the specimens in the italicized groups. Final groups incorporated into mixing triangle are shown.

	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Aquatic	4.5 ± 0.2	-29.1 ± 1.09
Berries	-4.0 ± 1.49	-26.9 ± 1.03
Terrestrial	-0.1 ± 1.69	-25.3 ± 1.43

Table 2. Potential diet items were sorted into three general groups and averaged(±SD). All $\delta^{15}N$ values were found to be significantly different using an independent t-test, $p<0.05$.

Common Name	Abbreviation	N	$\Delta^{15}N$	$\Delta^{13}C$
American Redstart	AMRE	19	1.9 ± 0.48	-26.38 ± 0.43
Ovenbird	OVEN	9	0.1 ± 0.61	-26.0 ± 0.38
Common Yellowthroat	COYE	3	1.8 ± 0.46	-26.7 ± 0.4
White-throated Sparrow	WTSP	3	0.8 ± 1.32	-26.8 ± 1.18
Black-and-white Warbler	BAWW	2	0.6 ± 0.85	-26.4 ± 0.43
Gray Catbird	GRCA	2	1.5 ± .39	-27.0 ± 0.44
Hermit Thrush	HETH	2	0.4 ± 0.02	-25.7 ± 0.39
Northern Waterthrush	NOWA	2	2.2 ± 0.54	-26.8 ± 0.55
American Robin	AMRO	1	1.6	-25.44
Black-capped Chickadee	BCCH	1	-0.5	-26.62
Hairy Woodpecker	HAWO	1	0.2	-24.98
Rose-breasted Grosbeak	RBGR	1	0	-26.6
Yellow Warbler	YEWA	1	2.5	-27.75
Yellow-bellied Sapsucker	YBSA	1	0.4	-25.41

Table 3. Sampled species' ^{13}C and ^{15}N isotope signatures with standard deviations. Whole blood isotope ratios adjusted with discrimination factors of heavier isotopes ($\delta^{15}N$-2.5‰, $\delta^{13}C$-2.0‰) reported by Herrera M. *et al.* (2006).

		Food Items		
Species	***N***	**Aquatic**	**Berries**	**Terrestrial**
Ovenbird	9	0.12-0.14	0.09-0.11	0.75-0.78
White-throated Sparrow	3	0.32-0.33	0.15-0.17	0.5-0.53
Black-and-white Warbler	2	0.23-0.25	0.08-0.1	0.65-0.68
Gray Catbird	2	0.41-0.43	0.07-0.09	0.48-0.51
Hermit Thrush	2	0.11	0	0.89
Black-capped Chickadee	1	0.2-0.21	0.33-.34	0.45-0.47
Rose-breasted Grosbeak	1	0.23-0.24	0.24-0.26	0.5-0.53
Yellow Warbler	1	0.61-0.62	0.05-0.08	0.3-0.34
American Redstart	16	NA		
Common Yellowthroat	3	NA		
Northern Waterthrush	2	NA		
American Robin	1	NA		
Hairy Woodpecker	1	NA		
Yellow-bellied Sapsucker	1	NA		

Table 4. Ranges of proportional contributions of each food item group to diets of 14 sampled species from the MAPS site near Douglas Lake in Northern Lower Michigan. Values show minimum-maximum ranges of contribution as calculated by the ISOSOURCE program (EPA 2007). NA indicates species ratio fell outside the ranges of food item averages.