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ABSTRACT

We sought to determine whether stable hydrogen isotope ratios can be used to estimate natal latitudes of Ruby-throated Hummingbirds (*Archilochus colubris*) as a way of examining migration patterns. Nine banders from across the Ruby-throated Hummingbird range in the U.S. collected r4 feathers from the first 10 hatching year (HY) birds banded in the 2003 season.The stable hydrogen isotope ratios for these feathers were determined, and these were regressed on latitude of collection site. A simple linear model explained 68% of the variation in isotope ratios, but an added quadratic expression boosted variation explained to 76%. However, the quadratic model does not differ significantly from the linear one. Hydrogen isotope ratios do not discriminate clearly among all southerly sites, but northerly latitudes show more variation. R4 feathers from every 20th HY bird banded in 2002 were collected in southern Illinois (n = 34). The skewed isotope distribution in this sample suggests the presence of migrants. Converting these 34 cases to t-scores based on the sample of the 10 first HY birds for southern Illinois suggests that three birds migrated from more northern latitudes, and 31 are likely from southern Illinois or nearby latitudes.

INTRODUCTION

Three main techniques used in studying bird migration are banding and recapture, using radio and satellite telemetry, and measuring hydrogen isotope ratios in feathers.The purpose of this exploratory study was to determine whether hydrogen isotope analysis is feasible and is a useful tool to study hummingbird migration.

Although hummingbird banding has an almost 30-year history in the United States, few articles are available on research about Ruby-throated Hummingbird (*Archilochus colubris*) migration.The limited published research on this species includes other diverse topics: cold hardiness in Canada (Floyd 1937), territoriality (Pitelka 1942), a note on observations (Saunders 1942), along with notes on chance sightings of hummingbirds made while making migration records of other birds (Saunders 1942, Van Tyne and Trautman 1945). More recently, there have been short communications published concerning counts of Ruby-throated Hummingbirds at Hawk Mountain Sanctuary in Pennsylvania (Willimont et al. 1988).

Banding is a good tool for discovering Ruby-throated Hummingbird site fidelity and survival rates of both young and adult birds, but not for studying long-distance migration. Each year a large number of hummingbirds are banded in southern Illinois during the breeding season and migration, totaling over 10,000 in six years (2000 - 2005). Recaptures at the initial banding site are common year after year, with 12.6% of the birds recaptured at the same site; however, foreign recaptures are quite rare. In six years of banding Ruby-throated Hummingbirds, foreign returns from birds banded in southern Illinois or birds

captured there but banded at other locales have occurred five times, once from Minnesota, twice from Pennsylvania, and twice from Texas. These five recaptures are not an effective basis for understanding migration patterns.

The use of radio collars and, especially recently, satellite telemetry has proven useful in studies of migration patterns of larger bird species, such as Thick-billed Murres (*Uria lomvia*), Tufted Puffins (*Fratercula cirrhata*; Hatch et al. 2000), and Corey's Shearwaters (*Calonectris diomedea*; Ristow et al. 2000). Satellite telemetry provides data on the precise movements of small samples of birds, pinpointing their successive locations over time, stopover spots, and related information. While radio collars can log data on movement with smaller areas (cf, Kjos and Cochran 1970), satellite telemetry is useful for studying both more local movements and seasonal migration between regions or continents. However, satellite telemetry devices have not yet reached the stage of miniaturization that can be used on hummingbirds.

We examined an alternative method for studying long-distance migration: detecting natal latitude by means of stable hydrogen isotope analysis in hatching-year (HY) birds' feathers. Recent research on other species has shown that stable isotope ratios can help to ascertain migration patterns (Hobson 1999, Hobson 2002, Hobson and Wassenaar 1997, Hobson et al. 2003, Meehan et al. 2001, Wassenaar and Hobson 2001).The ratio of deuterium to hydrogen atoms in precipitation varies systematically by latitude; and as nestling birds incorporate local diet, the hydrogen isotope ratio in their tissues mirrors that of local rainfall. A single feather can provide adequate material for the isotope analysis; however, only general latitudes of origin can be detected, not specific sites. For after-hatching-year (AHY) birds, this method could detect where new feathers were grown. Stable isotope measurement of feathers has proven to be useful in discovering migration distances and percentages of wintering birds from distant breeding populations (Hobson and Wassenaar 1997), in linking wintering and breeding areas for species (Hobson and Wassenaar 2001, Rubenstein et al. 2002), in studying altitudinal migration (Hobson et al. 2003), and determining the origins of birds caught at banding stations across North America (Wassenaar and Hobson 2001, Meehan et al. 2001). Studies of other fauna, such as monarch butterflies, using stable isotope measurements have shown a link between wintering colonies and the natal areas (Wassenaar and Hobson 1998, Hobson et al. 1999).

The model for the present study of Ruby-throated Hummingbirds is the study by Meehan et al. (2001) on HY Cooper's Hawks (*Accipiter cooperii*) migrating through the Florida Keys. In the Meehan study, feathers from HY Cooper's Hawks were analyzed to determine the birds' natal latitudes, thereby establishing links between migration and nesting regions. As in the study by Meehan et al. (2001), HY Ruby-throated Hummingbirds were chosen for this study because they grow their feathers while they are still in the nest and would, therefore, have retained the isotope marker from their natal region. Adult Ruby-throated Hummingbirds molt their contour feathers at the end of the breeding season and during migration (mostly July and August), and molt flight feathers in their wintering areas (Balltosser 1995), so for this study AHY birds were not suitable subjects because of the varying locales where their feathers are molted (i.e., anywhere along the migration route to its terminus).

METHODS

Feather Collection - From July through September of 2002, the r4 retrix was removed from a sample of every 20th HY Ruby-throated Hummingbird banded in southern Illinois (n = 34 feathers). From May to August of 2003, another baseline sample of feathers was collected by nine different banders across most of the breeding range of Ruby-throated Hummingbirds (New York, lat. = 43; Michigan, lat. = 42; Pennsylvania, lat. = 40; Missouri, lat. = 39; Illinois, lat. = 37; Oklahoma, lat. = 36; North Carolina, lat. = 35; Alabama, lat. = 32; and Louisiana, lat. = 30). Each bander collected the r4 feather from the first 10 HY birds banded in the year, reasonably guaranteeing that the HY birds were local birds and not migrants. These nine samples were gathered to determine whether isotope values vary by origin latitude for Ruby-throated Hummingbirds as they do for other species. Before migration could be examined, it is

important to see whether a strong gradient exists in the isotope ratios in Ruby-throated Hummingbird feathers from more northerly and southerly areas. These nine samples also provide baseline or comparison isotope values for distinguishing migrants from locally hatched Ruby-throated Hummingbirds in the southern Illinois sample of 34 feathers.

Stable Isotope Analysis - Feathers were cleaned in a 2:1 chloroform:methanol solvent rinse and prepared for stable-hydrogen isotope analysis at the National Water Research Institute in Saskatoon, SK. Stable-hydrogen isotope analyses of feathers are complicated due to the problem of uncontrolled isotopic exchange between samples and ambient water vapor (Wassenaar and Hobson 2000) but can be corrected using keratin standards as a means of correcting for this effect, so that the values reported here are equivalent to nonexchangeable feather hydrogen (Wassenaar and Hobson 2003). Briefly, the process involves the simultaneous measurement of unknowns with several replicates of three different keratin standards whose nonexchangeable δD values are known and which span the range of expected feather values. Algorithms generated from each run that relate δD values of unknowns to their expected nonexchangeable values are then used on a run-by-run basis.

Stable-hydrogen isotope measurements of feathers and keratin standards were performed on H_2 derived from high-temperature flash pyrolysis of feathers and continuous-flow isotope-ratio mass spectrometry (CF-IRMS). A Eurovector 3000™ (Milan, Italy) high temperature elemental analyzer (EA) with autosampler was used to automatically pyrolyse feather samples to a single pulse of H_2 gas. The resolved H_2 pulse was then introduced to the isotope ratio mass spectrometer (Micromass Isoprime™ with electrostatic analyzer) via an open split capillary. All δD results are expressed in the typical delta notation, in units of per mil (‰), and normalised on the Vienna Standard Mean Ocean Water – Standard Light Antarctic Precipitation (VSMOW-SLAP) standard scale. Repeated analysis of hydrogen isotope intercomparison material IAEA-CH-7 (-100‰), routinely included as a check, yielded an external repeatability of better than ±1.5‰. This method eliminates variation due to isotope exchange with ambient water vapor, but because not all laboratories follow this recommended standardization technique, our δD values may not be comparable to those reported by others. Based on within-run measurements of intercomparison material and consideration of within feather variance (Wassenaar and Hobson 2006), we estimate our laboratory error to be ±3 ‰.

RESULTS

The data were analyzed using the Statistical Package for the Social Sciences (SPSS). The first step in the analysis was to determine whether hydrogen isotope ratios were effective in studying Ruby-throated Hummingbird migration. If so, the isotope ratios of HY birds should vary by latitude.

Stage 1: Baseline Samples - The distribution of sample δD means by latitude does not appear to be linear (Fig. 1, 2). The southerly means (from southern Louisiana to northern Missouri) differ only slightly as latitude increases. Figure 1 shows medians and interquartile ranges. There is a gap in isotope ratio medians between latitudes 39° and 40° N (northern Missouri and Pennsylvania) that is larger than the differences between the southerly samples. The three northerly isotope ratio medians suggest a sharper level of change with higher latitude.

A difficulty in assessing the data is that there is an outlying case in each of the samples at 40° and 43° N latitude (Fig. 1). These two cases may contain observation or measurement errors that reduce the effectiveness of using isotopes to infer Ruby-throated Hummingbird migration. They could have been rejected statistically as outliers that are different from the otherwise homogeneous samples in which they occur, but are still used in this analysis. Along these same lines, the two most southerly samples show somewhat greater variation in isotope ratios than others, although there is no significant difference in variation among the nine sample means ($P > 0.05$). Care was taken to minimize measurement and recording error in the study, and the sources of this variation are not known.

Isotope ratios for the 90 samples were regressed on latitude, using a linear model and a quadratic model (Fig. 2). The best fitting straight line accounted for 68% of the variation in isotope ratios. The quadratic model accounted for an additional 8% of the total variation in isotope ratios, for a total of 76%. Nonetheless, the two models do not differ significantly; however, there is adequate co-variation demonstrated to infer natal latitudes in HY Ruby-throated Hummingbirds.

An analysis of variance shows more detail on the feasibility of inferring natal latitudes from isotope ratios in feathers. There was significant variation among sample means at the 95% confidence level; however, most sample means do not differ significantly from those of nearby latitudes. For example, the mean hydrogen isotope ratio for the Louisiana sample is not significantly different from those of Alabama, North Carolina, or Oklahoma, but is does differ significantly from all the samples from southern Illinois north. The Illinois and Missouri sample means differ from those from Louisiana and the three northern samples from 40° N latitude and above. Those sample means from 40° N and above do not differ significantly from each other, but do differ from all samples farther south. This analysis suggests that HY Ruby-throated Hummingbirds of northern origin, especially from 40° N or above, can be separated from those fledging further south by means of hydrogen isotope ratios.

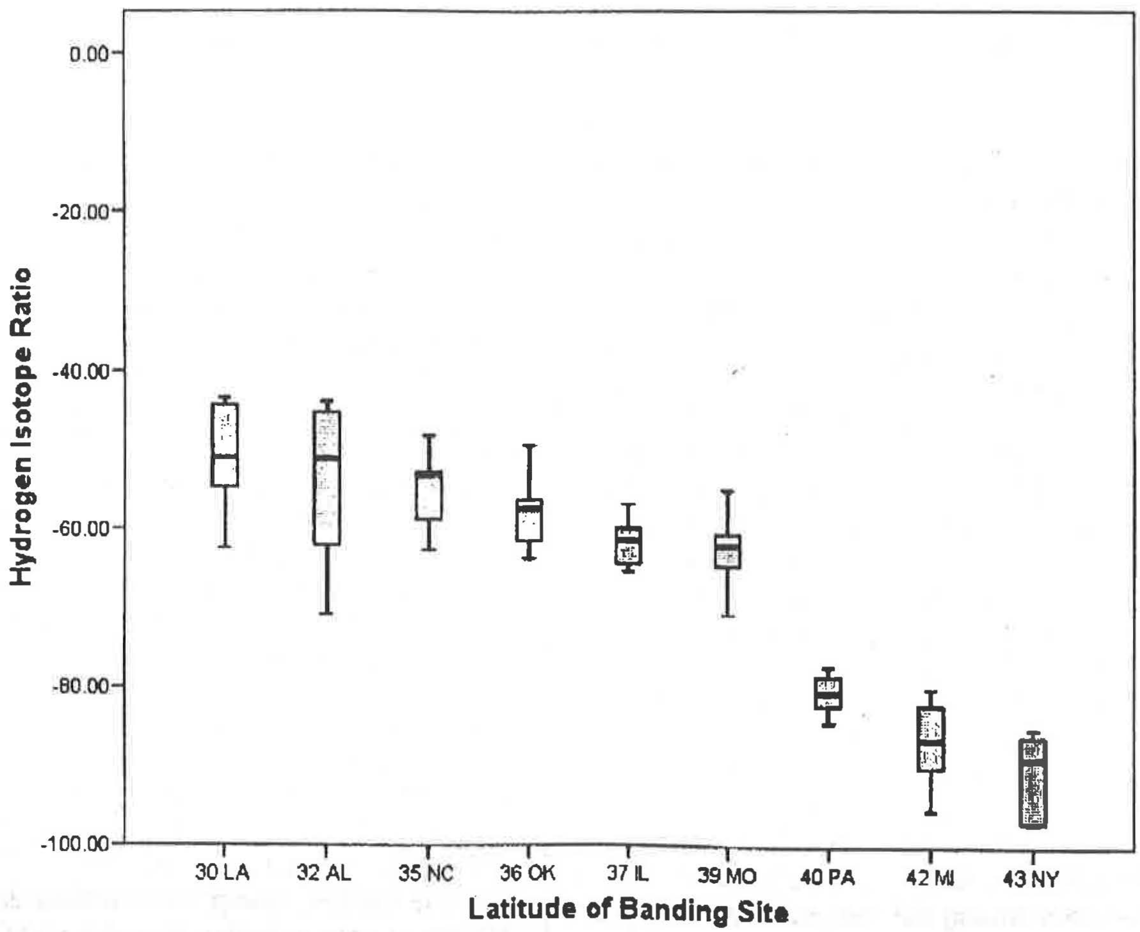

Fig. 1. Hydrogen isotope ratios in HY Ruby-throated Hummingbird feathers for nine samples (n = 10 for each). Boxes show the interquartile range. Asterisks for two northerly samples are extreme outlying cases detected by the SPSS graphing program.

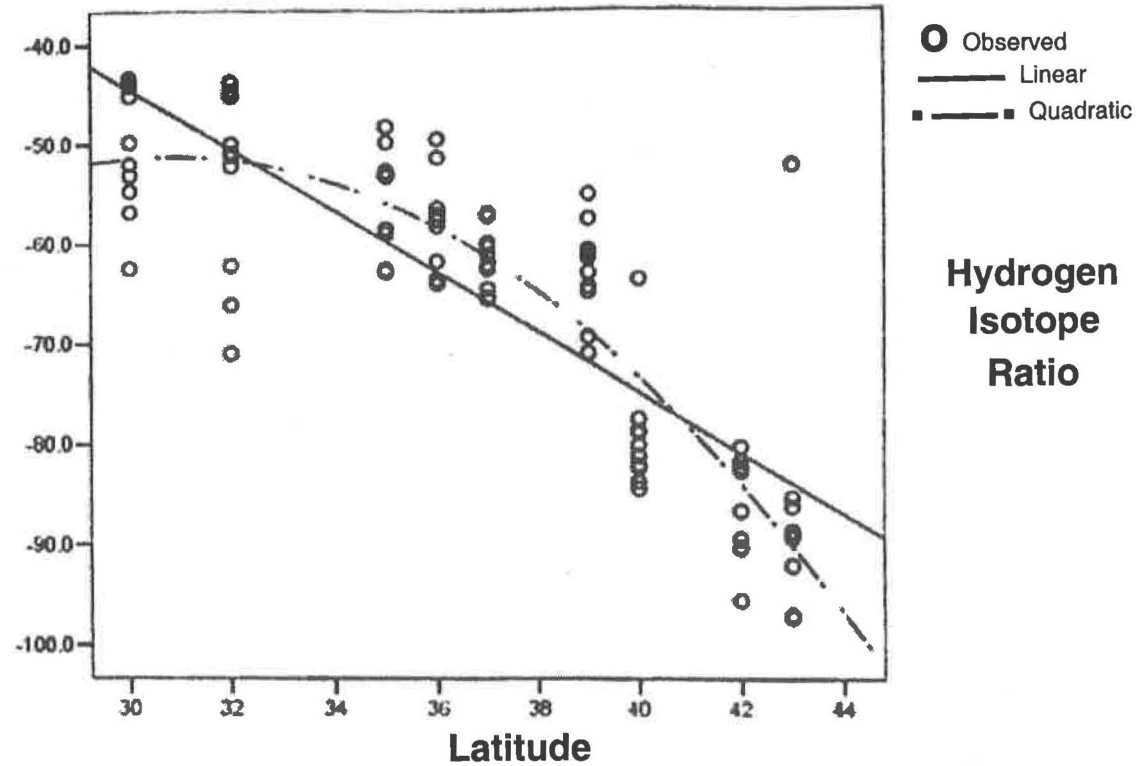

Fig. 2. Relationship of stable-hydrogen isotope ratio with latitude showing linear and quadratic regression line.

Stage 2 - Detecting migrants in a southern Illinois sample. – The second aspect of the research explores the use of hydrogen isotope ratios in detecting migrants within a season-long sample in 2002 of 34 HY Ruby-throated Hummingbirds in the southern Illinois counties of Union and Jackson. Since there may be small inter-annual differences in the weighted mean isotope concentrations in precipitation, the ratios for the 2002 sample may not be precisely comparable to those in the 2003 baseline sample. However, since the nine baseline samples do show systematic variation by latitude, the presence of numerous northern migrants in the season-long sample should skew the distribution somewhat and increase its variance relative to the baseline samples from the site. Migrants from more distant northern latitudes should therefore appear as statistical outliers. Assuming little variation in local isotope ratios among the two years, a confidence interval can be set up around the mean of the baseline sample and cases from the season-long sample compared to see whether they likely fall within that population.

The baseline sample mean is slightly lower than the season-long sample mean (-61 ‰ vs -59 ‰), but this is not a significant difference ($P = 0.47$). The season-long sample shows considerably more dispersion than the baseline sample, with standard deviations of 11.04 and 3.07, respectively. The Levene statistic yields a probability of only 0.06 that the two populations have equal variances. The box and whisker charts also show that the season-long sample is quite skewed (Fig. 3). Its mean is 1.02 standard deviations below the upper end of the distribution, but 3.76 standard deviations above the lower end. This suggests the presence of birds in the season-long sample that came from the north.

Three extreme statistical outliers detected by the SPSS program are located on the lower end of the distribution. To help ensure that we do not wrongly identify birds as migrants, we used a 99% confidence interval around the mean of the baseline sample. Using the standard deviation of the sample mean (rather than the usual standard error) enables the inference that 99% of the birds in the local population have isotope ratios between -71‰ and -52‰. Only the three extreme cases from the season-long sample fall below this confidence interval, suggesting that they migrated from the north.

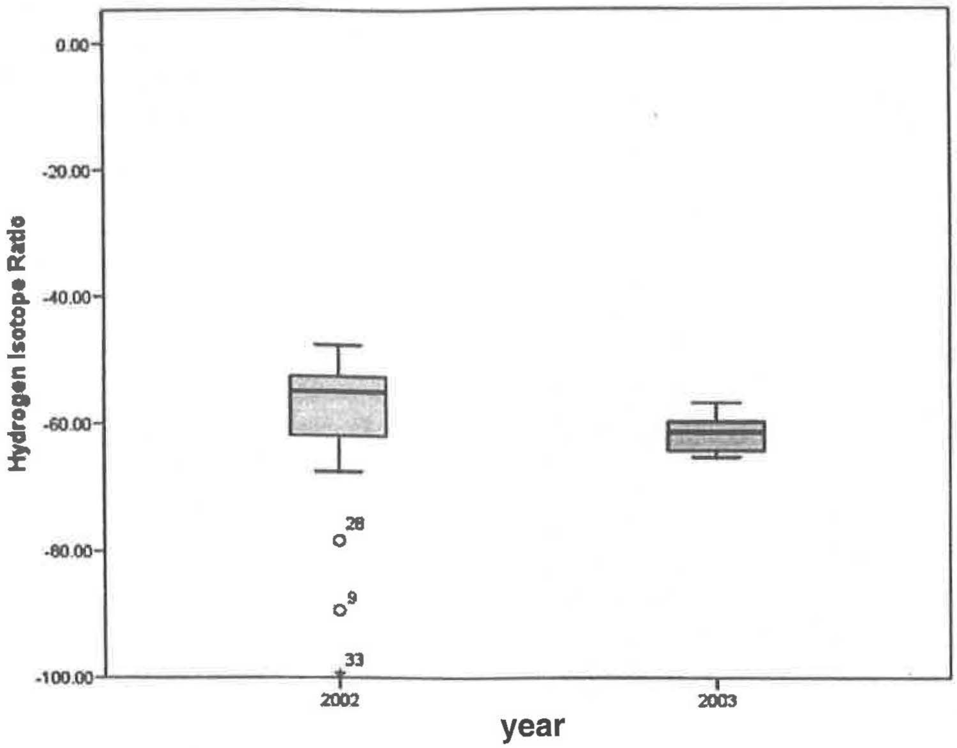

Fig. 3. Hydrogen isotope ratio of two southern Illinois samples, n = 34 for 2002 and n = 10 for 2003. Boxes show the interquartile range. Circles and asterisk for 2002 sample are extreme outlying cases detected by the SPSS graphing program. The numbers by these are case numbers assigned by SPSS

DISCUSSION

We suspect that we found few migrants because the local southern Illinois population is so abundant and that migrants are a small proportion of it. Local HY birds appear at feeders as early as 1 Jul, fledging from nests begun in mid- to late-April. In southern Illinois, females often complete a second nesting, with HY birds fledging from mid-August through the beginning of October. Most northern nesting Ruby-throated Hummingbirds are thought to complete only one nest cycle because of the shortness of summer. Given the number of Ruby-throated Hummingbirds in the study area of southern Illinois, where several hundred Ruby-throated Hummingbirds attend feeders at many houses and the potential of each female Ruby-throated Hummingbird to produce four young each year, the local population can easily overwhelm migrants.

The findings presented herein indicate that the use of stable isotope ratios may be applied to Ruby-throated Hummingbird migration. Because the data come from two different years and isotope ratios in precipitation vary by annual precipitation and temperature on a global basis, the feather hydrogen isotope ratios in the baseline samples and season-long sample from southern Illinois are not precisely comparable indicators of birds' natal location. Comparison of the isotope ratios of the three birds with the 95% confidence intervals from the analysis of variance of the nine baseline samples suggests that one fledged about 40° N, one about 42° N, and one above 43° N latitude–the location of our most northerly baseline sample.

The outliers from the more northerly locations that are seemingly from more southerly locations are of some concern. Differences in extent of feeder use among birds at a given location could produce variation in isotope ratios if water or sugar in feeders comes from a different latitude. A second possibility is northward migration after fledging. The first possibility can be explored by surveying the participating banders about the water and sugar sources at their banding locations, although a hummingbird is unlikely to use feeders in only one yard. In the next phase of the research we will replicate this study but with all samples from the same year and with a larger southern Illinois season-long sample to produce a better portrait of migration patterns.

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News, Notes, Comments

More Than Bird Banding

Besides my passion for bird banding, one of my interests over the years has been to prepare study skins for museums and an occasional live mount of birds and mammals for classrooms and nature centers. In the process, I teach my students how to prepare specimens in an informal weekly session called "specimen prep." I rely on the most complete and detailed reference on the subject (Winkler 2000).

I feel that the preparation of study skins is vital, as the addition of specimens to research museum collections have been diminished severely (Winkler 2000; Garrett pers. comm.) This has been due to two factors. One has been the general lack of training in this "art." Forty years ago at my institution (a community college), the preparation of a bird or a mammal as a study skin or a live mount was a "required" part of our introductory majors biology class. We no longer do this, and the preparation of study skins is barely taught in upper division ornithology and mammalogy courses. Another reason is that most biology students today would rather become molecular geneticists, and some are actually squeamish even to dissect a preserved frog. Third is that regulations and laws here in the United States and abroad, as well as the feelings of the general public, have changed over the years.

My specimens come from a variety of sources. Along with the occasional mortality from banding, I get specimens euthanized by rehab centers and zoos. I pick up road kills. Present and former students and colleagues are on the "lookout" for specimens for me as well as bringing me deceased pets. My college institution is considered an appropriate repository for such salvaged specimens, and I possess the necessary state and federal permits.

A while back, one of my banders brought in a group of eight Vaux's Swifts (*Chaetura vauxi*) that were found dead. As I was preparing them as study skins, I noted that one had an extra toe (polydactyly) asymmetrically located on each foot. This is not exactly a rare phenomenon, as it is normal in some breeds of chickens, occasionally found in humans, and unfortunately more common in amphibians (Sakai 2006). Yet, I could only find 10 other avian examples from the literature and from museums.

This brings me to the juxtaposition of preparing study skins and bird banding. I then asked myself whether I would have been observant enough to notice this oddity if I happened to be banding this bird. Since that incident, I have alerted my banders to be aware. A few years ago, the banders at Point Reyes Bird Observatory were observant enough to note that a House Wren *(Troglodytes aedon)* that they caught had an extra pair of rectrices (Humple 1999). This point is emphasized again in an accompanying note in this issue by the banders at Tortuguero in Costa Rica who noted a bifurcated rectrix (Burton and Froelich 2007). The lesson is: keep an eye out for such anomalies and be ready to document (photograph) and take copious notes on any such phenomenon.