Waterbirds around the world

A global overview of the conservation, management and research of the world's waterbird flyways

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First published in 2006 by The Stationery Office Limited
71 Lothian Road, Edinburgh EH3 9AZ, UK.

Applications for reproduction should be made to Scottish Natural Heritage,
Great Glen House, Leachkin Road, Inverness IV3 8NW, UK.

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

ISBN 0 11 497333 4

Recommended citation:

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Using stable isotope ratios to unravel shorebird migration and population mixing: a case study with Red Knot Calidris canutus


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ABSTRACT
Identifying demographic mechanisms is fundamental to understanding the causes of population change in waterbirds. This may be relatively easy for static breeding and wintering populations, but populations of mixed breeding or wintering origin often occur in stopover sites in spring and autumn, and thus estimates of survival and recruitment from these areas are inevitably representative of all the birds marked, rather than individual populations. We used stable isotope analysis of flight feathers to identify the different wintering populations of Red Knot Calidris canutus rufa that passed through Delaware Bay, north-eastern USA, in the springs of 2004 and 2005. Here, they feed and fatten on an abundance of Horseshoe Crab Limulus polyphemus eggs before flying to their Arctic breeding areas. δ13N values separated birds from wintering areas in southern South America (“southern” birds) and Brazil/south-eastern USA (“northern” birds). Northern birds were further separated using δ13C values. Approximately 55% of the birds caught within Delaware Bay were from the southern population, 22.5% from Brazil, and 12.5% from the south-eastern USA, while 10% were of unknown (although most likely “northern”) origin. At a site on the Atlantic coast of Delaware Bay, where only Mussel Mytilus spp. spat were available, the proportion of short-distance migrants from the south-eastern USA was much higher, and is most likely related to their shorter-hop migration strategy that allows them to take advantage of this hard-shelled prey resource.

INTRODUCTION
The migration of shorebirds is one of the most inspiring and impressive spectacles in the natural world. The Red Knot Calidris canutus is a flagship species among long-distance shorebird migrants. In the Americas, populations winter in the south-eastern USA, in northern Brazil and the Caribbean, and at sites in Tierra del Fuego and Patagonia. In order to reach breeding grounds in the Canadian Arctic, some individuals make an annual round-trip of 30 000 km. For these birds, Delaware Bay is the last major northward staging site where they join hundreds of thousands of other shorebirds to feed on the eggs of Horseshoe Crabs Limulus polyphemus. During a 10-14 day stopover, Red Knots increase their body mass by over 70%, gaining the fuel for their final non-stop flight to the breeding grounds.

Over the past five to ten years there has been a dramatic reduction in the numbers of Red Knot passing through Delaware Bay (Baker et al. 2004), with similar declines noted in the South American wintering areas (Morrison et al. 2004) but apparently not in the relatively poorly known population wintering in the south-eastern USA (Niles et al. in prep.). For a species that makes such long distance migrations to tight deadlines (Piersma et al. 2005), there are many potential pressure points in the annual cycle.

One of the major aims of a study, initiated in 1997, is to determine the causes of the change in the Red Knot populations of the West Atlantic Flyway by marking individuals and using survival and recruitment models (White & Burnham 1999) to estimate demographic rates. However, one of the problems of studying birds on passage sites is that individuals from several different populations may mix. Ideally, demographic rates would be estimated for each population separately, thereby enabling better understanding of the issues under study. This requires a means of assigning individuals to different populations, which in the case of shorebirds is often difficult owing to no single clear distinguishing feature. Morphometrics and genetic markers can sometimes partly or wholly distinguish different subspecies of shorebirds (Wenink

Using carbon and nitrogen stable isotopes in flight feathers from a pilot sample of 100 individual Red Knots sampled in Delaware Bay in spring 2003, Atkinson et al. (2005) showed that birds from at least four different wintering populations passed through Delaware Bay in spring 2003. The basic technique involves measuring isotope ratios in feathers moulted in known wintering areas, then deriving comparable figures from individuals caught in Delaware Bay so that they can be assigned with confidence to one of the wintering areas. This paper develops and applies this method to 1,220 and 947 individual Red Knots caught during spring staging in Delaware Bay in 2004 and 2005, respectively. We determine the proportion of birds originating from the major wintering areas, and seek to give guidance on the use of stable isotopes in migration studies.

METHODS
FEATHER SAMPLING AND ISOTOPE ANALYSIS
To determine the wintering area of birds caught in Delaware Bay, we took a sample of the sixth primary covert, measured ascendently, from each bird caught. This feather was used because it is moulted at the time of the birds main primary moult and is likely to be indicative of the main wintering area (for full details see Atkinson et al. 2005). Each feather was washed in a solution of 0.25M sodium hydroxide to remove dirt and grease, rinsed thoroughly in distilled water, then dried overnight in an oven at 75˚C. Each sample was finely chopped using surgical scissors into pieces no longer than 2 mm in length, and between 0.5 and 1 mg of each feather was accurately weighed into tin capsules and loaded into an automatic sampler. Stable isotope ratio measurements of carbon and nitrogen were made using CF-IRMS (Continuous Flow Isotope Ratio Mass Spectrometry). All stable isotope values are reported in permil (‰) using the delta (δ) notation:

$$\delta \text{ isotope} = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000$$

where δ isotope is the sample isotope ratio (13C or 15N) relative to a standard (traceable to a primary international standard), and R is the ratio of heavy to light isotopes (13C/12C or 15N/14N) in the sample or standard. Δ13C and Δ15N are reported relative to their primary international standards, namely PeeDee Belemnite (V-PDB) and atmospheric nitrogen (V-AIR), respectively. Routine measurements were precise to within 0.1‰ for δ13C and 0.3‰ for δ15N.

STATISTICAL ANALYSIS
First, a small number of sub-adult birds were identified by having a carbon isotope signature (δ13C < -19.5‰) typical of the freshwater systems where their feathers were grown. These birds were therefore hatched in the previous summer and were approximately 10-11 months old. A two-stage process was then used to estimate the origins of adult birds caught in Delaware Bay in 2004 and 2005: first, birds were divided into northern or southern winterers; second, northern winterers were divided into those of USA or Brazilian/Caribbean origin.

Atkinson et al. (2005) showed that there was a clear division in the values of δ15N between birds wintering in Patagonia and

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<td>2003/2004</td>
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<td>2004/2005</td>
<td>-15.3 ± 1.03</td>
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<td>-16.58 ± 0.41</td>
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Table 1. δ13C values of primary coverts of adult Red Knot Calidris canutus known to belong to the two northern wintering populations based either on birds caught or individually-marked birds observed in the wintering areas. Winter refers to the boreal winter in which the coverts were grown.

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Tierra del Fuego (δ15N > 13.5‰, termed “southern” birds) and elsewhere (δ15N < 13.5‰, termed “northern” birds). Northern birds comprise two geographically distinct populations, in the southern-eastern USA and Brazil, but the isotope signatures of birds from each overlap. We therefore calculated the mean and SD of the δ13C values of birds known to winter in each area from birds either caught there (33 birds caught in Maranhão State, Brazil, in November 2004 and February 2005) or observations of known individuals which had been sampled the previous spring in Delaware Bay (Table 1). To estimate the proportion of individuals originating from each wintering area, we assumed that the distribution of δ13C values of northern birds caught in Delaware Bay was a composite of two overlapping normal distributions, one of δ13C values from Brazilian birds and the other of δ13C values from birds from the south-eastern USA. We used a least squares method to fit two normal curves (based on the means and SDs of δ13C values of birds from known wintering areas) to the observed frequency distribution of the δ13C values from birds in Delaware Bay. δ15N values were not used, as there was little difference between the two means. We excluded any outlying “northern” type birds that had δ13C or δ15N values more than 2 SD from either of the two known reference sample means, and classed these birds as unknown. The proportion of birds from each wintering area was adjusted until the overall sum of squares of the observed minus estimated numbers for each δ13C category was minimized. As isotope values may differ annually, year-specific values of δ13C were used. This method gave a means of estimating the relative proportion of birds from Brazil versus the south-eastern USA, but did not permit individual northern birds to be allocated to one of these populations, although it was, of course, possible to assign the probability of belonging to each population based on the δ13C values.

RESULTS AND DISCUSSION
Origin of the birds passing through Delaware Bay in 2004 and 2005
In both 2004 and 2005, stable isotope ratios of individual Red Knots separated well (Fig. 1) according to the divisions used by Atkinson et al. (2005). Sub-adult birds (i.e. birds < 1 year old) made up a small proportion of the total birds caught in Delaware Bay, with 28 individuals in 2004 and 31 in 2005. Averaged across the two years, 51.8% of the adult birds were from the southern population (Tierra del Fuego/Patagonia) and 48.2% were from the northern population (south-eastern USA/Brazil and other as yet unknown areas). Approximately 20% of northern adults deviated by >2 SD from the δ13C and δ15N means of birds from known wintering areas (Fig 1, box) and

Waterbirds around the world
Fig 1. Plots of δ¹³C and δ¹⁵N values of Red Knot Calidris canutus caught in Delaware Bay in (a) spring 2004 and (b) spring 2005. Southern birds refer to the population wintering in Patagonia and Tierra del Fuego; northern, to those wintering in north-western Brazil, the Caribbean and the south-eastern USA.
were labelled as of unknown origin. Decomposition of the frequency distribution of δ13C values of the remaining northern birds then divided into approximately 45:55 south-eastern USA to Brazil (Fig. 2). These translate into proportions of the Delaware passage population as 21.1% from Brazil, 17.6% from the south-eastern USA and 9.6% unknown, with mostly small differences between years (Fig. 1, Table 2). There was one major difference in the composition of the catches between years. More birds with a south-eastern USA origin were caught in 2005 than in 2004, due to a change in catching locations. In 2004, all individuals were caught within the confines of the bay itself, whereas in 2005, catches were also made on the beaches and marshes on the Atlantic coast of Delaware Bay. These latter catches contained a much higher proportion of the shorter-distance migrants from the south-eastern USA.

Prior to this study, it was originally thought that the majority of the birds passing through Delaware Bay were from the populations wintering in Tierra del Fuego and Patagonia. However, the stable isotope data indicate that in 2004 and 2005 only approximately half of the individuals were from there.

**Use of stable isotopes in studies of birds on passage sites**

These results show that the use of stable isotopes can be an extremely useful tool for assigning individual birds to distinct populations in cases where these populations have distinct stable isotope signatures. In this case, birds from very different geographical areas (northern and southern groups) were distinguishable. As a tool for population biologists, this is extremely useful because it may help to explain the basis of heterogeneity in survival and re-sighting rates, and opens the door to calculating population-level demographic parameters from mixes of populations. For instance, in coming years it will be possible to evaluate survival, recruitment, passage times and staging behaviour for specific Red Knot wintering populations, thus helping to elucidate the causes of declines and best management for recovery.

To use stable isotopes successfully in this way, it is necessary to collect reference material from known wintering areas, and this is labour-intensive if the species in question migrates to many different areas. Fortunately in this case, Red Knot are known to winter in a small number of well-known and well-studied sites, although even for this species, around 10% of the passage birds in Delaware Bay were classed as unknown. This is not to say that they are necessarily from different wintering locations, but these unknowns could be birds adopting a different diet or moultting at a different time of year. Indeed they might be two year old birds. Isotope ratios vary temporally in response to, for example, changing seasons, and Red Knot in their first year of life do not return to the breeding grounds the following summer. Instead, they remain on or near the wintering areas and tend to undergo a full primary moult several months earlier than the adults returning from the breeding areas. Although poorly understood at present, this is likely to be the explanation for the “unknown” northern signatures and will be the subject of further study.

As well as discriminating between locations separated by large geographical distances, isotope ratios may change over a relatively small scale. Isotope signatures from Red Knot known to winter in Bahia Lomas in Chile and Rio Grande in Argentina,
approximately 160 km apart, are found to be very different (Atkinson et al. 2005). This is most likely due to the fact that the city of Rio Grande is at the head of the estuary and discharges from the city are likely to alter $\delta^{13}$C and $\delta^{15}$N values in the sediments surrounding the estuary. In this case, such differences are useful. This also prompts caution in applying broad-scale isotopic gradients to large areas without knowledge of the processes operating on the ground.

This method relies on birds growing feathers or other tissues in areas with predictably different isotope ratios. For species that are more widespread in winter, or use similar habitats in different locations, such clear-cut results may not be achieved. For example, in a study of the American Golden Plover Pluvialis dominica and Pacific Golden Plover P. fulva, feathers grown in the wintering grounds showed no differences in $\delta^D$, $\delta^{13}$C and $\delta^{15}$N values between species, despite wintering on different continents (Rocque 2003).

These results also show that some isotope ratios are prone to annual fluctuations, probably related to climate. Thus, although the threshold $\delta^{15}$N and $\delta^{13}$C values used to distinguish northern and southern birds, and adults and sub-adults (still with juvenile primaries), appear to be robust from year to year, there were more marked annual differences in the south-eastern USA/Brazil distinction. This suggests that samples should be collected over several years to help evaluate natural variation in isotope signatures and limit its impact on misclassification of individuals. Analysis of additional isotopes may also help classification, but may be prohibitively expensive.

**Linking breeding and wintering locations**

In certain circumstances, stable isotopes allow researchers to draw links throughout the flyway of individual species. Provided the necessary reference samples have already been discriminated, samples from adults on the breeding grounds may enable direct linkages to be drawn between breeding and wintering locations. However, for high Arctic breeders such as the Red Knot, sampling feathers from juveniles on the wintering grounds to identify breeding grounds is less productive. This is because there appear to be few consistent and predictable geographic patterns in isotope ratios in the largely terrestrial/freshwater tundra habitats. For lower latitude breeders, differences may be more apparent where large-scale differences in geology or primary production exist. For instance, the strontium signature found in bones of the Common Redshank Tringa totanus can be used to distinguish birds from Iceland, with a relatively young geology, from those breeding in Scotland, where rocks are much older (Evans 2004).

In conclusion, stable isotopes coupled with colour-marking birds as individuals can offer a useful way of distinguishing mixed populations of shorebirds on passage sites. In situations similar to Delaware Bay, their usage offers the opportunity of calculating parameters such as survival, recruitment, mass gain, stopover time etc. for populations that winter many thousands of kilometres apart.

**ACKNOWLEDGEMENTS**

We would like to thank all the volunteers who helped out with the shorebird banding programmes in Argentina, Chile and Delaware Bay. We are grateful to the U.S. Fish and Wildlife Service and National Environment Research Council (NERC) for funding the isotope analyses, and would like to acknowledge Greg Breese for his help and support of the project. The field-work was funded, in part, through a grant from the Delaware Division of Fish and Wildlife with funding from the Division of Federal Aid, U.S. Fish and Wildlife Service, under State Wildlife Grants appropriation 05020201-2330. Stable isotope analyses were carried out at the NERC Life Sciences Mass Spectrometry Facility under a grant from NERC, by Dr Alina Marca at the Stable Isotope Laboratory at the University of East Anglia and by Dr Tom Maddox at the University of Georgia.
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