

## TRACE ELEMENTS HAVE LIMITED UTILITY FOR STUDYING MIGRATORY CONNECTIVITY IN SHOREBIRDS THAT WINTER IN ARGENTINA

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**Abstract.** Trace-element analysis has been suggested as a tool for the study of migratory connectivity because (1) trace-element abundance varies spatially in the environment, (2) trace elements are assimilated into animals' tissues through the diet, and (3) current technology permits the analysis of multiple trace elements in a small tissue sample, allowing the simultaneous exploration of several elements. We explored the potential of trace elements (B, Na, Mg, Al, Si, P, S, K, Ca, Ti, Cr, Mn, Ni, Cu, Zn, As, Sr, Cs, Hg, Tl, Pb, Bi, Th, and U) to clarify the migratory connectivity of shorebirds that breed in North America and winter in southern South America. We collected 66 recently replaced secondary feathers from Red Knots (*Calidris canutus*) at three sites in Patagonia and 76 from White-rumped Sandpipers (*C. fuscicollis*) at nine sites across Argentina. There were significant differences in trace-element abundance in shorebird feathers grown at different nonbreeding sites, and annual variability within a site was small compared to variability among sites. Across Argentina, there was no large-scale gradient in trace elements. The lack of such a gradient restricts the application of this technique to questions concerning the origin of shorebirds to a small number of discrete sites. Furthermore, our results including three additional species, the Pectoral Sandpiper (*C. melanotos*), Wilson's Phalarope (*Phalaropus tricolor*), and Collared Plover (*Charadrius collaris*), suggest that trace-element profiles change as feathers age. Temporal instability of trace-element values could undermine their application to the study of migratory connectivity in shorebirds.

**Key words:** annual variability, contamination, *Calidris*, migration, spatial variation.

### Limitada Utilidad de los Elementos Traza para Determinar Conectividad Migratoria en Chorlos que Pasan la Temporada No-reproductiva en Argentina

**Resumen.** El análisis de elementos traza ha sido sugerido como herramienta para estudiar la conectividad migratoria por las siguientes razones: (1) la abundancia de elementos traza varía espacialmente en el ambiente, (2) los elementos traza son incorporados en los tejidos animales a través de la dieta, y (3) avances tecnológicos permiten el análisis de múltiples elementos traza a partir de una pequeña muestra de tejido. Aquí exploramos el potencial de los elementos traza (B, Na, Mg, Al, Si, P, S, K, Ca, Ti, Cr, Mn, Ni, Cu, Zn, As, Sr, Cs, Hg, Tl, Pb, Bi, Th y U) para estudiar la conectividad migratoria en aves costeras que se reproducen en América del Norte y que pasan la temporada no-reproductiva en el sur de América del Sur. Se colectaron plumas secundarias recientemente reemplazadas de dos especies de *Calidris* en sus sitios no-reproductivos; 66 de *C. canutus* de tres sitios en la Patagonia y de 76 de *C. fuscicollis* de nueve sitios en Argentina. Observamos diferencias significativas en las abundancias de elementos traza entre las plumas que fueron mudadas en sitios distintos, y la variación anual en un sitio fue pequeña comparada con la variación entre sitios. Los elementos traza muestran un patrón espacial relacionado a la geología de los sitios muestreados, pero no observamos un gradiente a través de la Argentina (excepto para mercurio). La falta de dicho gradiente limita el uso de esta técnica sólo a preguntas sobre el origen de las aves entre un pequeño número de posibles orígenes. Más aun, incorporando otras tres especies, *C. melanotos*, *Phalaropus tricolor* y *Charadrius collaris*, nuestros resultados sugieren que al envejecer las plumas, la abundancia de elementos traza cambia. La inestabilidad temporal en los valores de los elementos traza puede ser una limitante importante para su aplicación en el estudio de la conectividad migratoria en aves costeras.

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## INTRODUCTION

Migratory connectivity, defined as the cross-seasonal link between the breeding and nonbreeding grounds of a migratory species (Webster et al. 2002), has important evolutionary, ecological, and conservation implications (Webster and Marra 2005). Biochemical markers, such as stable isotopes and trace elements, are useful for studying connectivity because they occur in natural environments, their abundance varies spatially (Hanson and Jones 1976), they are assimilated into animals' tissues through water and diet (Kelsall and Burton 1977, Bortolotti and Barlow 1988), and are fixed in some tissues that become metabolically inert after growth ceases (e.g., hair, feathers, and nails) (Hanson and Jones 1976, Bortolotti and Barlow 1985). Stable isotopes have been used as markers extensively and have proven useful in determining migratory connectivity in butterflies, fish, birds, and mammals (e.g., Vogel et al. 1990, Wassenaar and Hobson 1998, McCarthy and Waldron 2000, Kelly et al. 2002, Rubenstein and Hobson 2004). Far less work has been done in exploring the potential of trace elements as biochemical markers, however, despite some promising initial results (Hanson and Jones 1976, Kelsall and Burton 1977, Parrish et al. 1983, Burger et al. 2001, Szép et al. 2003, Norris et al. 2007, Kaimal et al. 2009).

Since the application of trace elements to identifying the site of birds' molt was first proposed, it has been limited by the difficulty of making precise measurements (Devine and Peterle 1968, Kelsall 1970, Hanson and Jones 1976, Bortolotti et al. 1990, Wenzel and Gabrielsen 1995). Recently, however, the analysis of trace elements has become more accessible to researchers through advances in technology (Ridley and Lichte 1998, Ridley 2000, Kaimal et al. 2009). Trace-element analysis has several advantages that make it an attractive tool for studying migratory connectivity. First, current technology permits the analysis of multiple trace elements in very small tissue samples (Ridley and Lichte 1998, Ridley 2000). Second, this analysis allows for the simultaneous exploration of several elements independently (Norris et al. 2007). Finally, trace elements may vary across small geographic scales, allowing a high degree of spatial resolution (Szép et al. 2003).

In spite of these advantages, there are potential problems with trace elements in the study of migratory connectivity. First, the abundance of trace elements in feathers grown at the same site may vary from year to year (Kelsall and Burton 1979, Bortolotti and Barlow 1988). Second, many elements show no large-scale spatial pattern (Donovan et al. 2006), potentially reducing the application of the technique to species with a limited distribution (Norris et al. 2007, Szép et al. 2009). Finally, there is some evidence that feathers' chemical profiles are not stable after their growth is completed (Rose and Parker 1982, Edwards and Smith 1984, Bortolotti et al. 1988).

In this paper, we evaluate the utility of trace elements for the identification of the nonbreeding grounds of nearctic shorebirds. The consequences of our evaluation rest on one

key assumption: newly grown secondary flight feathers carry the chemical signature of the sites where birds are captured. For this assumption to be valid, shorebirds must have been faithful to the capture site during the molt, and the diet consumed during feather growth must be reflected in the trace-element composition of feathers (which seems to be justified, at least for stable isotopes; Hobson and Clark 1992). If this assumption is valid, trace elements might be of utility in identifying the location of individual shorebirds' nonbreeding grounds because surficial geology varies spatially (Parker et al. 1997; Fig. 1), implying variation in the abundance of chemical elements (Font et al. 2007, Sellick et al. 2009). For trace elements to be useful in this context, however, four require-

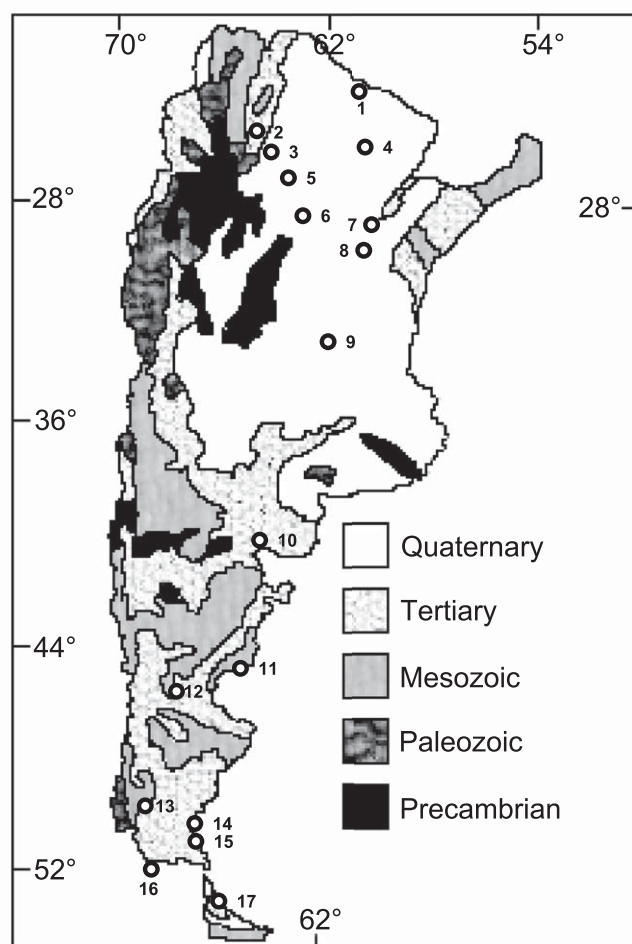


FIGURE 1. Spatial pattern of geological regions in Argentina, with newest regions in the northeast (lighter color) and older regions in the south and west (darker colors). The sites sampled in this study cover a wide range of latitude and include multiple geological regions. 1, Bañados de la Estrella; 2, El Cadillal; 3, Río Hondo; 4, La Limpia; 5, Bañados de Figueroa; 6, Arroyo Saladillo; 7, Arroyo Aguilar; 8, Bajos Submeridionales; 9, Bañados de Saladillo; 10, San Antonio Oeste; 11, Bahía Bustamante; 12, Laguna Grande; 13, Laguna Nimez; 14, Punta Loyola; 15, Laguna Río Gallegos; 16, Bahía Lomas; 17, Río Grande. (Modified from Parker et al. 1997).

ments must be met: (1) trace-element profiles in feathers must vary predictably by site, (2) annual variation at a site must be smaller than the variation among sites, (3) most of the variation must be explained by changes in space (i.e., changes in latitude and longitude or differences between geological regions), and (4) the chemical profile of a feather must remain constant as the feather ages. We evaluated each of these four requirements specifically.

## METHODS

### STUDY SPECIES AND SAMPLING SITES

We selected the Red Knot (*Calidris canutus*) and the White-rumped Sandpiper (*C. fuscicollis*) as focal species because their patterns of geographic distribution in South America differ. The subspecies of Red Knot (*C. c. rufa*) that we studied spends the nonbreeding season in southern South America, in coastal Patagonia of Argentina and Chile and in smaller numbers in Venezuela and Brazil (Piersma and Davidson 1992). The White-rumped Sandpiper spends the nonbreeding season in southern South America, where it is widely distributed across most of Argentina east of the Andes to Uruguay, Brazil, and Paraguay (Parmelee 1992). To determine if trace-element profiles in feathers are stable through time, we also analyzed feather samples from the Pectoral Sandpiper (*C. melanotos*), Wilson's Phalarope (*Phalaropus tricolor*), and Collared Plover (*Charadrius collaris*); the last of these breeds in South America. The nonbreeding distribution of the Pectoral Sandpiper overlaps that of the White-rumped Sandpiper, except in southern Patagonia, where the Pectoral Sandpiper does not occur. Wilson's Phalarope is widely distributed as a winter visitor from Peru to Patagonia (Hayman et al. 1986). The Collared Plover ranges from Mexico to southern Argentina (Hayman et al. 1986).

We trapped shorebirds in mist nets and cannon nets during the period of wing-feather molt between late December and early February, 2002–2004. We obtained 76 samples (between 4 and 24 per site) of recently replaced feathers of the White-rumped Sandpiper from nine sites across Argentina (sites 8–17 in Fig. 1) and 66 samples (between 10 and 40 per site) of the Red Knot from three sites in Patagonia and Tierra del Fuego (sites 10, 16, and 17 in Fig. 1). In addition, we captured 10 Collared Plovers, 9 Pectoral Sandpipers, and 2 Wilson's Phalaropes in northern Argentina (sites 1–9 in Fig. 1).

### FEATHER SAMPLING

The species with which we worked molt their primaries and secondaries simultaneously, although the primary molt is typically more advanced. In all cases we had several feathers from which to choose. Bortolotti and Barlow (1985) found variation in trace-element profiles within and among feathers of a single individual. The major differences were between different types of feathers (i.e., flight vs. body feathers), but there is no evidence of, nor is there any a priori expectation of, systematic differences among individual flight feathers

for physiological reasons. Therefore, to reduce variability, we sampled only secondaries, and to increase the probability that the sampled feathers were characteristic of the collection site, we collected the most recently grown secondary from each individual. Sampled secondary feathers were thoroughly washed in a 2:1 chloroform: methanol solution, dried overnight in a fume hood, and stored in the laboratory in small plastic bags. Although this method of cleaning is effective in eliminating most of the surface contaminants from feathers, it is possible that some particles were not effectively removed (Font et al. 2007), potentially introducing a source of error in our estimation of the absolute abundances of trace elements, but this error should be consistent for all feathers so should not affect our conclusions.

### TRACE-ELEMENT ANALYSIS

All samples were processed in the U.S. Geological Survey (USGS) Laser Ablation Laboratory in Denver, Colorado. To determine the abundance of 24 chemical elements, we processed the samples by laser ablation with a New Wave UP213 system laser coupled to a PE Sciex Elan 6000 mass spectrometer. We selected these elements,  $^{11}\text{B}$ ,  $^{23}\text{Na}$ ,  $^{25}\text{Mg}$ ,  $^{27}\text{Al}$ ,  $^{29}\text{Si}$ ,  $^{31}\text{P}$ ,  $^{34}\text{S}$ ,  $^{39}\text{K}$ ,  $^{44}\text{Ca}$ ,  $^{47}\text{Ti}$ ,  $^{52}\text{Cr}$ ,  $^{55}\text{Mn}$ ,  $^{60}\text{Ni}$ ,  $^{63}\text{Cu}$ ,  $^{66}\text{Zn}$ ,  $^{75}\text{As}$ ,  $^{88}\text{Sr}$ ,  $^{133}\text{Cs}$ ,  $^{202}\text{Hg}$ ,  $^{205}\text{Tl}$ ,  $^{208}\text{Pb}$ ,  $^{209}\text{Bi}$ ,  $^{232}\text{Th}$ , and  $^{238}\text{U}$ , because they occurred at measurable levels in a preliminary sample of shorebird flight feathers. We ran the laser along the feather's rachis in a line 1500  $\mu\text{m}$  long and 40  $\mu\text{m}$  wide at a speed of 10 mm sec $^{-1}$  with a pulse rate of 10 Hz and an energy rate of 80%. This sampled line was confined to the middle third of the feather shaft because a preliminary sample showed that this region was much more homogeneous with regard to element concentrations than the base or the tip of the feather. Values are expressed in parts per million (ppm), except for those of  $^{11}\text{B}$ ,  $^{23}\text{Na}$ ,  $^{75}\text{As}$ ,  $^{202}\text{Hg}$ , and  $^{209}\text{Bi}$ , which are expressed as abundance relative to the internal laboratory Micro-Analytical Organic Standard (MAOS, USGS; Ridley and Lichte 1998, Ridley 2000).

### STATISTICAL ANALYSIS

To determine if chemical profiles of feathers varied by site, we analyzed the data for the Red Knot and White-rumped Sandpiper separately. For these analyses we used only sites from which we had more than five samples. We compared the spatial distribution of profiles of each element with ANOVA, or with Kruskal–Wallis one-way ANOVA if the data were not normal even after a log or square-root transformation. To determine if the differences among sites were large enough for them to be distinguished on this basis, we used the stepwise discriminant function of linear discriminant analysis (LDA). The results presented here correspond to the “leave-one-out cross validation” or “jack-knifed” method, which consists of excluding a sample from the data set, estimating the discriminant functions, and then assigning the excluded sample on

the basis of those functions. Furthermore, we tested the discriminant functions for the White-rumped Sandpiper with 10 feathers from Laguna Nimez that were randomly selected and removed prior to the analysis. In this exercise we considered these 10 feathers as of “unknown origin.”

To determine if variability between years within a site was smaller than variability among sites we used the data for the Red Knot. This data set contains samples from 2 successive years from Río Grande (2002, 2003) but samples from Bahía Lomas and San Antonio Oeste from 2003 only. Using LDA, we generated discriminant functions first on the basis of Río Grande 2002 plus the other two sites, then on the basis of Río Grande 2003 only plus the other two sites. Then we used the functions for 2002 to assign samples from 2003 and functions for 2003 to assign samples from 2002. If the variability between years within a site was smaller than the variability among sites, we expected the function to assign the Río Grande samples to the Río Grande group independently of the year on which the model was based (e.g., no Río Grande samples would be assigned to Bahía Lomas or San Antonio Oeste).

To test whether trace-element profiles have a large-scale spatial pattern, we analyzed all White-rumped Sandpiper samples, including sites with fewer than five samples to increase the range of latitudes and longitudes covered by this dataset. We analyzed the data in two ways, first, by quadratic discriminate analysis (QDA) to determine how well samples could be assigned to their geologic regions (Fig. 1), using a jack-knife classification approach. We set as equal the prior probabilities of a sample being assigned to each of the regions. Additionally, we expected that there might be longitudinal/latitudinal gradients in some trace elements because in Argentina the geological age of the surface decreases as one moves eastward away from the Andes. Therefore, our second analysis of the spatial pattern of each element was a regression analysis in a general linear model. The full model included latitude, longitude, the interaction of both, and a quadratic term for both variables. We removed terms one by one, selecting for removal the term with the lowest *t*-test statistic. Single terms (e.g., latitude and longitude) were kept in the model even when not significant if a major term including it was significant.

Finally, we tested whether a feather's chemical profile changed with time with a multiple-species data set from northern Argentina (50 individuals of four species from nine sites). We used a paired *t*-test to compare the trace-element profiles of freshly grown secondaries to the profiles of one-year old secondaries of the same individual. If data were not normally distributed, we transformed them with the natural logarithm or the square root; if normality was not achieved with these transformations, then we applied the Wilcoxon signed-ranks test. For this analysis, we had to assume that one-year-old feathers were grown at the same site as fresh feathers. To maximize the likelihood that this assumption was valid, we removed certain individuals from the analysis. We eliminated juvenile *Calidris*

sandpipers and phalaropes from the analysis because their old feathers would have been grown in the Arctic. We identified juveniles by their plumage characteristics and feather deuterium values: arctic deuterium signatures are substantially depleted compared to Argentine values (Argentina range: -85 to 7‰; arctic feathers <-115‰; unpubl. data).

## RESULTS

### DO TRACE ELEMENTS' PROFILES VARY BY SITE?

To test this question we analyzed samples of the White-rumped Sandpiper and Red Knot. In the White-rumped Sandpiper, 12 elements out of 24 varied significantly by site (Table 1). The pattern of differences was not consistent, however (Fig. 2). The best LDA model assigned samples to origin with a general error rate of 0.18; error rates for each site ranged from 0 to 0.5 (Table 2). The best LDA model included five elements

TABLE 1. Variability among sites and spatial pattern of variation in the abundance of 24 elements in feathers of White-rumped Sandpipers wintering in Argentina<sup>a</sup>.

|                   | Kruskal-Wallis | Regression model   | <i>F</i>  | <i>r</i> <sup>2</sup> |
|-------------------|----------------|--|-----------|-----------------------|
| <sup>11</sup> B   | 49.312***      | lat + long + lat × long + lat <sup>2</sup> + long <sup>2</sup> | 4.640***  | 0.195                 |
| <sup>23</sup> Na  | 34.746***      | lat + long   | 9.470***  | 0.184                 |
| <sup>25</sup> Mg  | 19.647**       | lat + long   | 3.127†    | 0.054                 |
| <sup>27</sup> Al  | 10.528         | —  | —         | —                     |
| <sup>29</sup> Si  | 6.764          | —  | —         | —                     |
| <sup>31</sup> P   | 15.053*        | —  | —         | —                     |
| <sup>34</sup> S   | 6.960          | lat + lat <sup>2</sup>   | 5.158**   | 0.100                 |
| <sup>39</sup> K   | 18.492**       | lat + long + lat * long + lat <sup>2</sup> + long <sup>2</sup> | 2.538*    | 0.093                 |
| <sup>44</sup> Ca  | 8.525          | —  | —         | —                     |
| <sup>47</sup> Ti  | 9.531          | —  | —         | —                     |
| <sup>52</sup> Cr  | 7.635          | —  | —         | —                     |
| <sup>55</sup> Mn  | 3.960          | —  | —         | —                     |
| <sup>60</sup> Ni  | 9.531          | —  | —         | —                     |
| <sup>63</sup> Cu  | 6.479          | —  | —         | —                     |
| <sup>66</sup> Zn  | 18.062**       | lat + lat <sup>2</sup>   | 5.707**   | 0.112                 |
| <sup>75</sup> As  | 16.507**       | —  | —         | —                     |
| <sup>88</sup> Sr  | 36.590***      | long + long <sup>2</sup>                                       | 5.380**   | 0.105                 |
| <sup>133</sup> Cs | 12.414*        | lat + long   | 3.636*    | 0.066                 |
| <sup>202</sup> Hg | 34.534***      | lat + long + lat * long + lat <sup>2</sup> + long <sup>2</sup> | 12.378*** | 0.431                 |
| <sup>205</sup> Tl | 3.956          | lat + lat <sup>2</sup>   | 3.588*    | 0.065                 |
| <sup>208</sup> Pb | 12.571*        | lat + long + lat * long + lat <sup>2</sup>                     | 5.342***  | 0.188                 |
| <sup>209</sup> Bi | 27.180***      | lat + long + lat * long + long <sup>2</sup>                    | 7.563***  | 0.259                 |
| <sup>232</sup> Th | 8.137          | —  | —         | —                     |
| <sup>238</sup> U  | 10.805         | —  | —         | —                     |

<sup>a</sup>Symbols coding levels of probability: †*P* < 0.1, \**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001.



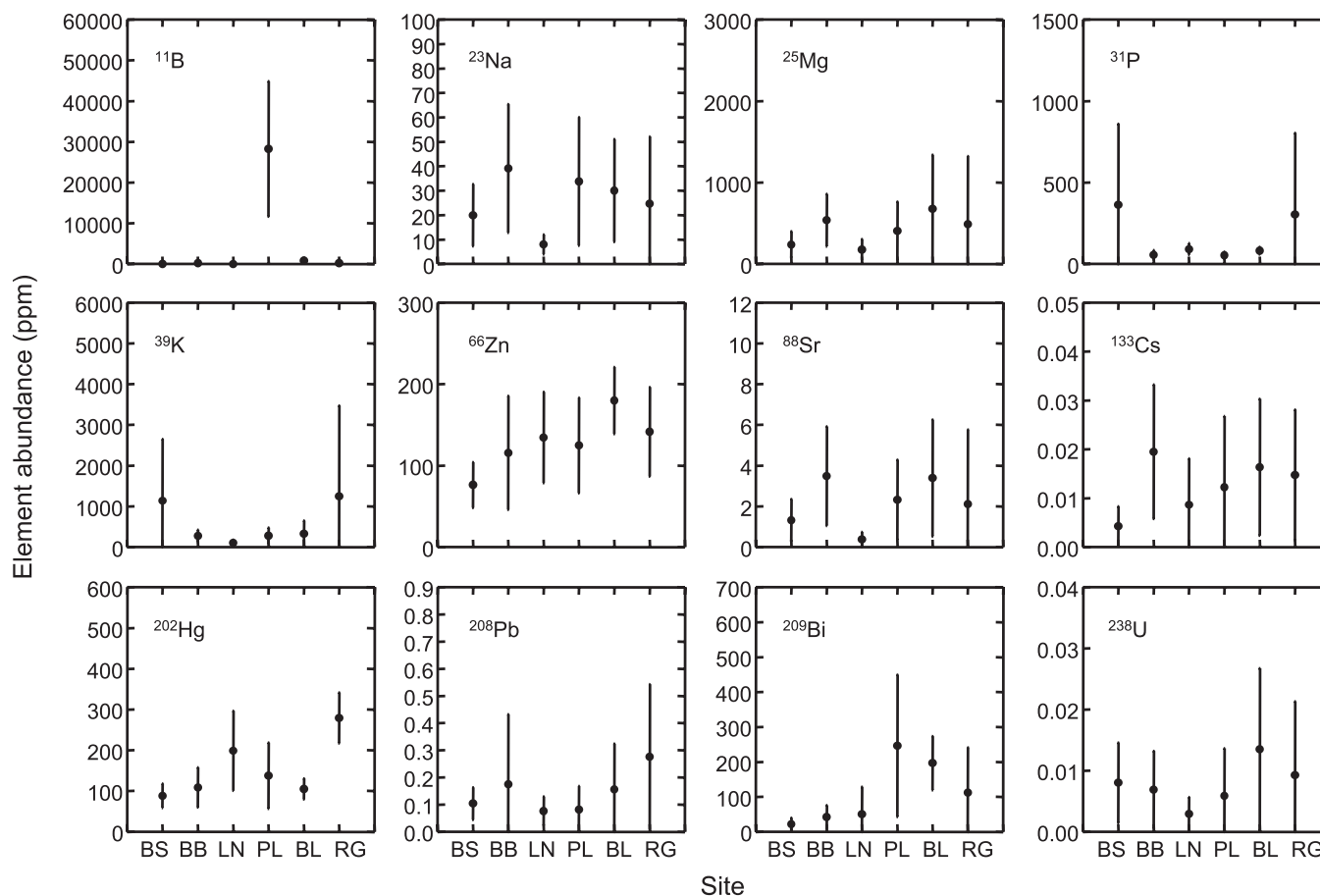


FIGURE 2. Mean ( $\pm$ SD) concentration of 12 trace elements that varied significantly by site in feathers of the White-rumped Sandpiper collected on the nonbreeding grounds. The sites are ordered along the x-axis by latitude, with north to the left. Note that the elements' patterns differ and that there is no systematic change with latitude (BS, Bañados de Saladillo; BB, Bahía Bustamante; LN, Laguna Nimez; PL, Punta Loyola; BL, Bahía Lomas; RG, Río Grande).

that did not differ significantly by site as discriminating variables (Tables 1 and 2). Of the 10 feathers of "unknown origin" from Laguna Nimez, 8 were correctly assigned, and the other 2 were assigned to Río Grande.

To analyze Red Knot samples we considered the samples from Río Grande in 2002 and 2003 separately. Thus, we first compared Bahía Lomas and San Antonio Oeste to Río Grande 2002 and then to Río Grande 2003. When we considered only Río Grande 2002 samples, only  $^{11}\text{B}$  ( $F_{2,44} = 35.35$ ,  $P < 0.001$ ),  $^{31}\text{P}$  ( $F_{2,44} = 3.27$ ,  $P = 0.05$ ),  $^{34}\text{S}$  ( $F_{2,44} = 5.86$ ,  $P < 0.01$ ), and  $^{208}\text{Pb}$  ( $F_{2,44} = 4.83$ ,  $P = 0.01$ ) differed significantly by site. On the other hand, when we considered Río Grande 2003 samples, only  $^{11}\text{B}$  ( $F_{2,42} = 7.99$ ,  $P < 0.001$ ) and  $^{75}\text{As}$  ( $F_{2,42} = 3.38$ ,  $P = 0.04$ ) differed significantly by site. Despite the lack of variation among sites in most elements, LDA models were very good at assigning samples to the correct origin, using again as discriminant variables some elements that did not show differences among sites. When only samples from Río Grande 2002 were included, the best model assigned samples to their

origin with a general error rate of 0.09 (site-specific error rates: Bahía Lomas, 0.19; Río Grande 2002, 0.05; San Antonio Oeste, 0). The discriminant variables in this model were  $^{11}\text{B}$ ,  $^{23}\text{Na}$ ,  $^{25}\text{Mg}$ ,  $^{31}\text{P}$ ,  $^{34}\text{S}$ ,  $^{47}\text{Ti}$ ,  $^{52}\text{Cr}$ ,  $^{88}\text{Sr}$ , and  $^{209}\text{Bi}$ . On the other

TABLE 2. Error rates of leave-one-out cross-validation assignment of White-rumped Sandpiper feathers collected on the non-breeding grounds. Discriminant variables are  $^{11}\text{B}$ ,  $^{23}\text{Na}$ ,  $^{29}\text{Si}$ ,  $^{31}\text{P}$ ,  $^{34}\text{S}$ ,  $^{44}\text{Ca}$ ,  $^{52}\text{Cr}$ ,  $^{55}\text{Mn}$ ,  $^{66}\text{Zn}$ ,  $^{88}\text{Sr}$ , and  $^{202}\text{Hg}$ .

| Site                   | <i>n</i> | Error rate |
|------------------------|----------|------------|
| Bahía Bustamante       | 8        | 0          |
| Bahía Lomas            | 6        | 0.33       |
| Bañados del Saladillo  | 8        | 0.12       |
| Laguna Nimez           | 14       | 0.14       |
| Laguna Nimez "unknown" | 10       | 0.20       |
| Punta Loyola           | 8        | 0.12       |
| Río Grande             | 8        | 0.50       |
| Total                  | 62       | 0.18       |

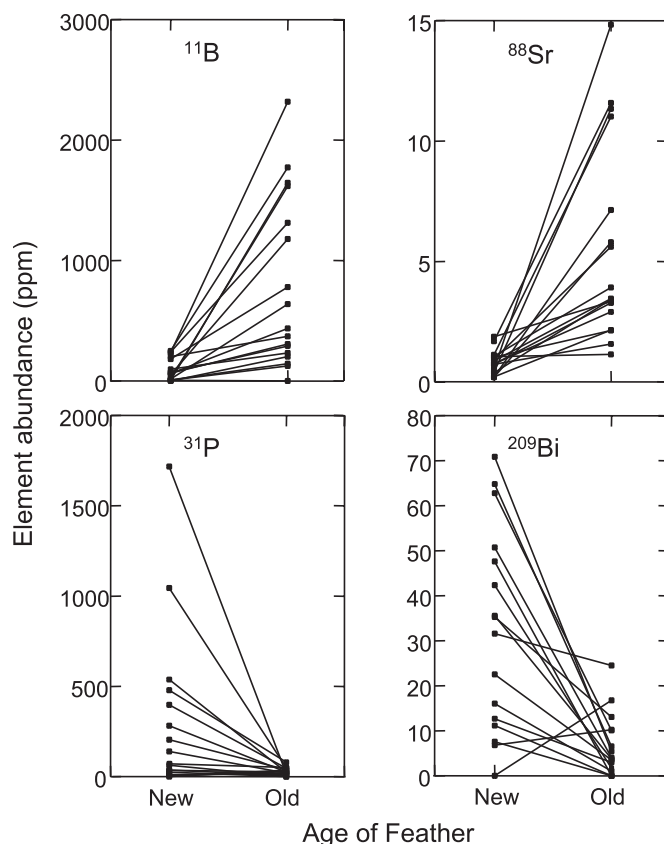


FIGURE 3. Comparison of concentrations of four trace elements in freshly grown feathers and one-year-old feathers. The two upper panels exemplify how concentrations of some elements increase with a feather's age, while the lower two panels exemplify how the concentrations of others decrease with a feather's age. Deuterium values showed indirectly that the feathers did not differ in origin.

hand, the general error rate increased to 0.29 (site-specific error rates: Bahía Lomas, 0.31; Río Grande 2003, 0.21; San Antonio Oeste, 0.4), when only samples from Río Grande 2003 were included in the analysis. In this case the best discriminant variables were  $^{11}\text{B}$ ,  $^{27}\text{Al}$ ,  $^{31}\text{P}$ ,  $^{52}\text{Cr}$ , and  $^{75}\text{As}$ .

One of the potential advantages of trace-element analysis is that it allows for the inclusion of several elements, some of which may not be correlated. We found that most elements are not strongly correlated. Nonetheless,  $^{23}\text{Na}$  was correlated with 15 other elements (all  $P < 0.01$ ,  $r > 0.4$ ), and  $^{27}\text{Mg}$ ,  $^{44}\text{Ca}$  and  $^{88}\text{Sr}$  were correlated with nine elements (all  $P < 0.01$ ,  $r > 0.4$ ). All other elements were correlated with four to eight other elements, except  $^{60}\text{Ni}$ ,  $^{202}\text{Hg}$ , and  $^{52}\text{Cr}$ , which were not correlated with any other element, and  $^{11}\text{B}$ , which was correlated only with  $^{209}\text{Bi}$ .

#### IS ANNUAL VARIABILITY WITHIN A SITE LARGER THAN VARIABILITY AMONG SITES?

In the comparison the Red Knot samples collected at Río Grande in 2002 to those collected at the same site in 2003, the abundances of only  $^{11}\text{B}$  ( $t_{23,9} = 3.12$ ,  $P < 0.01$ ) and  $^{60}\text{Ni}$  ( $t_{20,1} =$

2.28,  $P = 0.03$ ) differed.  $^{11}\text{B}$  was included as a discriminant variable in the construction of both LDA models (see above), yet  $^{60}\text{Ni}$  was not included in either of them.

In the attempt to assign samples of the Red Knot to site of origin with the discriminant functions from the model based on samples from Río Grande 2002, only 11 of 19 samples from Río Grande 2003 were assigned to Río Grande. Seven of these samples were assigned incorrectly to Bahía Lomas, one to San Antonio Oeste. The discriminant functions from the model based on the 2003 samples, however, correctly assigned 19 of 21 samples from Río Grande 2002 to Río Grande. This difference is interesting because the 2002 model was the one with the lower error rate. The 2003 model performed poorly (see above) but was better at assigning "unknown" feathers (i.e., feathers not used to construct the model). From these analyses, we conclude that there is some level of annual variation in some elements. Nonetheless, the results suggest that annual variation within a site is smaller than variability among sites.

#### DO TRACE ELEMENTS' PROFILES SHOW A LARGE-SCALE SPATIAL PATTERN?

White-rumped Sandpiper samples were available from only three of the geological regions, but a combination of nine elements as discriminant variables ( $^{11}\text{B}$ ,  $^{23}\text{Na}$ ,  $^{34}\text{S}$ ,  $^{39}\text{K}$ ,  $^{47}\text{Ti}$ ,  $^{52}\text{Cr}$ ,  $^{66}\text{Zn}$ ,  $^{202}\text{Hg}$ , and  $^{238}\text{U}$ ), classified samples to their region of origin with a general error rate of 0.13 (range 0.04–0.25). However, we found no significant gradient with respect to latitude or longitude. Concentrations of several of the analyzed elements varied significantly, but in general these patterns explained only a small portion of the variation among sites (Fig. 2, Table 1). The only exception is  $^{202}\text{Hg}$ , for which 43% of the variability was explained by changes in latitude and longitude, including the interaction between these and quadratic terms for both variables. Values of  $^{202}\text{Hg}$  were lowest in the northeast and the southwest of the study area and increased toward both the northwest and southeast. These results suggest that there may be a large-scale pattern that may be useful for identifying geographic origin for this species, but if so it is more complex than a simple latitudinal/longitudinal gradient.

#### DO TRACE ELEMENTS' PROFILES CHANGE WITH A FEATHER'S AGE?

For this analysis we used four species, and we pooled feathers from several sites in northern Argentina. From an initial sample of 50 individuals for which we had both old and fresh secondaries, 30 were juveniles that were discarded from the analysis because their old feathers were grown at their site of hatching in the Arctic. The data were not normally distributed even after transformation, so we present results of Wilcoxon signed-rank tests only. Freshly grown and one-year-old feathers differed significantly in 12 of the 24 elements analyzed (Table 3). Figure 3 depicts how the profiles of four selected elements changed.  $^{11}\text{B}$  was the most important

TABLE 3. Mean differences in chemical profiles between new and one-year old feathers of shorebirds in northern Argentina. The last three columns show the *F*-values of elements used in linear-discriminant-analysis models to assign Red Knot and White-rumped Sandpiper feathers to origin of molt.

| Element           | Mean difference <sup>a</sup> | Z-test <sup>b</sup> | Value of <i>F</i> from LDA models <sup>c,d</sup> |                 |                 |
|-------------------|------------------------------|---------------------|--|-----------------|-----------------|
|                   |                              |                     | White-rumped Sandpiper                           | Red Knot (2002) | Red Knot (2003) |
| <sup>11</sup> B   | -506.91                      | -3.070**            | 24.24  | 33.95           | 11.38           |
| <sup>27</sup> Al  | -50.05                       | -3.724***           |  |                 | 7.29            |
| <sup>29</sup> Si  | 248.71                       | -2.366*             | 2.96   |                 |                 |
| <sup>31</sup> P   | 288.43                       | 3.027**             | 4.01   | 7.40            | 2.97            |
| <sup>44</sup> Ca  | -737.47                      | -3.201**            | 7.17   |                 |                 |
| <sup>47</sup> Ti  | -3.03                        | -3.724***           |  | 2.99            |                 |
| <sup>55</sup> Mn  | -4.13                        | -3.332***           | 3.68   |                 |                 |
| <sup>88</sup> Sr  | -6.75                        | -3.724***           | 3.59   | 6.65            |                 |
| <sup>208</sup> Pb | -0.70                        | -2.243*             |  |                 |                 |
| <sup>209</sup> Bi | 35.11                        | 2.635**             |  | 4.14            |                 |
| <sup>232</sup> Th | -0.01                        | -2.330*             |  |                 |                 |
| <sup>238</sup> U  | -0.09                        | -3.724***           |  |                 |                 |

<sup>a</sup>Mean of new feathers minus mean of old feather.

<sup>b</sup>Symbols coding levels of probability: \**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001.

<sup>c</sup>Only *F*-values for those elements that showed an effect of age are shown.

<sup>d</sup>Red Knot (2002) is the model constructed on the basis of samples from Río Grande in 2002; Red Knot (2003) is the one constructed on the basis of samples from Río Grande in 2003.

variable in all three of the LDA models, and <sup>88</sup>Sr increased with a feather's age (Fig. 3, upper panel). Seven other elements also followed this pattern of increase. <sup>31</sup>Phosphorus and <sup>209</sup>Bi decreased in abundance (Fig. 3, lower panel), as did <sup>29</sup>Si (Table 3). These results suggest that trace-element profiles do, in fact, change with a feather's age.

## DISCUSSION

On the basis of these analyses, we conclude that trace-element analyses of feathers are potentially useful for discriminating among sites of origin of shorebird feathers. Although the analyses showed some correlation among some elements, several trace elements in feathers were weakly or uncorrelated. Differences among sites in feathers' chemical profiles are not consistent in all elements, although differences among sites resulted in low error rates when samples were assigned to origin with linear discriminant models. More importantly, 8 out of 10 samples of White-rumped Sandpipers of "unknown origin" were correctly assigned, suggesting that trace elements have the potential to be useful in the study of migratory connectivity in shorebirds.

Furthermore, we found that (except for <sup>11</sup>B and <sup>60</sup>Ni) chemical profiles in Red Knot feathers from Río Grande in 2002 did not differ significantly from those in 2003. When feathers from Río Grande in 2002 were assigned with the 2003 model, most of the samples were correctly assigned to Río Grande, suggesting that at Río Grande variability among

sites is still higher than variability between years. The error rate was higher when 2003 samples were assigned with the model based on 2002 samples, although either model assigned most of the samples correctly. Annual variation in trace-element profiles can be associated with changes in diet from year to year (Kelsall and Burton 1977, 1979, Szép et al. 2003), so the estuary used by Red Knots at Río Grande may not be that variable, and similar items are available each year, reducing annual variability (Szép et al. 2003). Hence our results for the Red Knot cannot be assumed to apply to other species in other areas, as has been shown by other authors who have found annual variation in their particular situations (Kelsall and Burton 1979, Bortolotti and Barlow 1988).

In spite of these promising results, however, our results also identified potential limitations on the application of trace-element profiles to the study of migratory connectivity in shorebirds wintering in Argentina. We found that White-rumped Sandpipers can be accurately assigned to broad geological regions (Fig. 1), and we were able to assign both Red Knots and White-rumped Sandpipers to a small number of known, discrete sites. For the selected elements, however, we found no large-scale spatial gradient in Argentina. The only exception to this result was <sup>202</sup>Hg, for which 43% of the variation was explained by changes in latitude and longitude. The lack of any large-scale spatial gradient will prevent accurate assignment for species such as the White-rumped Sandpiper, whose feathers can originate from a large number of unknown sites across Argentina.

A second and perhaps more significant issue in applying trace elements to determine migratory connectivity is the possibility of chemical profiles changing as a feather ages (Rose and Parker 1982, Bortolotti et al. 1988). We found indirect evidence that the abundance of some elements in shorebird feathers changed after the feather was grown. This result is perhaps not surprising, given that it has been suggested that feathers could be used to measure rates of atmospheric deposition of heavy metals in polluted areas (Furness 1993). The chemical composition of feathers could change as the result of direct contact with dirt, preening, or chemical leaching (Edwards and Smith 1984, Goede and deBruin 1986, Bortolotti et al. 1988), and different elements may change at different rates because of differences in deposition rate and the strength of bonding with feathers' keratin. Our results are not unique in this regard; several other studies have also found that chemical contamination of feathers, at least with metals, is common (Rose and Parker 1982, Edwards and Smith 1984, Goede and deBruin 1986, Bortolotti et al. 1988).

We do not know with certainty that the individuals whose feathers we analyzed molted at the same site in successive years, or that the abundance of trace elements at these sites was the same in successive years. The apparent changes that we noted could simply reflect annual variability in trace elements' abundance within a site or annual variability in the location of molt. However, we found no significant variability in trace-element profiles of Red Knot feathers collected at Río Grande in two years (see above). Furthermore, we think that change of the site of molt in successive years is a poor explanation for the patterns that we observed. We observed consistently similar changes in the same direction, for multiple trace elements, in four species of shorebirds, and at several molting sites. That such consistent changes are due to random changes in molting sites is highly unlikely and is much more likely due to changes with feather aging. The temporal changes that we found are consequential for identifying the location of molt because nine of the 12 elements that changed through time were selected as discriminant variables in at least one of our LDA models, and two of them were selected in all three models (Table 3).

In summary, our study suggests that the application of trace elements in feathers to studies of migratory connectivity in birds should be approached carefully. The lack of a large-scale gradient of variation in trace-element abundance limits the application of this technique for shorebirds wintering in Argentina. The technique may be useful to assign samples to a small number of known origins. But, even when only a small number of origins are possible, values of trace elements can be misleading because chemical profiles of feathers may change with time.

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