MIGRATION STRATEGIES OF WINTERING POPULATIONS OF RED KNOTS *CALIDRIS CANUTUS RUFA* IN SOUTH AMERICA: THE ROLE OF PARASITE PRESSURE

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**SUMMARY.**—Migration strategies of wintering populations of red knots *Calidris canutus rufa* in South America: the role of parasite pressure.

**Aims:** To test whether different migratory strategies in red knots (*Calidris canutus rufa*) are a response to spatial variation in parasite pressure at different marine wintering sites as predicted by the ‘parasite’ hypothesis of Piersma (1997).

**Location:** Río Grande and Tierra del Fuego, Argentina; Maranhão, Brazil; Delaware Bay, USA.

**Methods:** The abundance of ectoparasites and blood parasites and the frequency of parasitized birds were estimated at the three sites. Ectoparasites were scored visually and parasites in blood were searched for with molecular assays and observations of smears on slides. Birds caught in mixed flocks refueling in Delaware Bay were assigned to either northern (Maranhão or possibly Florida) or southern wintering sites (Tierra del Fuego) using stable isotopes in feathers.

**Results:** All ectoparasites found were feather lice were Mallophaga, Phthiraptera. The 4.4 % of birds in Tierra del Fuego and the 100 % in Maranhão had ectoparasites. In Delaware Bay the proportion of parasitized birds from northern and southern sites was not significantly different. No blood parasites (*Plasmodium* spp., *Haemoproteus* spp. and *Leucocytozoon* spp.) were found in the samples.

**Conclusions:** Only 4.4 % of birds wintering in Tierra del Fuego had ectoparasites whereas all birds wintering in Maranhão were parasitized, often heavily. During migration through Delaware Bay in May, the proportion of parasitized birds from northern (50 %) and southern (40.1 %) sites was not significantly different, indicating that many southern birds had been infected during a short stopover on the northwards migration or by direct contact in Delaware Bay. The parasite hypothesis predicts that red knots should evolve migrations to low-parasite marine wintering sites to reduce the fitness consequences of high ectoparasite load in tropical Maranhão, but there is likely to be a tradeoff with increased mortality for long-distance migration to cold-temperate Tierra del Fuego. All blood parasite assays were negative for *Plasmodium* spp., *Haemoproteus* spp. and *Leucocytozoon* spp, consistent with the low incidence of blood

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parasite vectors in marine shores. Observations in each wintering site support a role for parasite pressure in the evolution of migration strategies in red knots. However, it will be very important to extend studies to birds captured at Delaware Bay since results could suggest that southern populations would have detrimental effects of longer migratory distances and ectoparasites load, whereas northern populations only would suffer the negative effects of the parasite load. The results open the way for similar tests of the parasite hypothesis on other pathogens and endoparasites.

**Key words**: red knots, wintering sites, ectoparasites, blood parasites.

**RESUMEN.—Estrategias de migración de la población invernante de correlimos gordo Calidris canutus rufa en Sudamérica: la importancia de la presión parasitaria.**

**Objetivos**: Comprobar si las diferentes estrategias de migración en correlimos gordo *Calidris canutus rufa* responden a la variación especial en la presión parasitaria en diferentes localidades marinas de invernada, como predice la hipótesis de los parásitos propuesta por Piersma (1997).

**Localidad**: Río Grande y Tierra de Fuego, Argentina; Maranhão, Brasil; Bahía de Delaware, Estados Unidos.

**Métodos**: Se estimó la abundancia de ectoparásitos y parásitos sanguíneos y la frecuencia de aves parasitadas en tres localidades. Los ectoparásitos fueron estimados visualmente y los parásitos sanguíneos se analizaron con técnicas moleculares y con observaciones de frotis sanguíneos. Se asignó el área de invernada de las aves capturadas en Bahía de Delaware mediante análisis isotópicos de las plumas: zonas norte (Maranhão o posiblemente Florida) y sur (Tierra del Fuego).

**Resultados**: Los ectoparásitos encontrados fueron parásitos de las plumas (Mallophaga, Phthiraptera). El 4.4 % de las aves en Tierra del Fuego y el 100 % en Maranhão presentaron ectoparásitos. En Bahía de Delaware la proporción de aves con ectoparásitos procedentes el norte y de sur no fue significativamente diferente. No se encontraron parásitos sanguíneos (*Plasmodium* spp., *Haemoproteus* spp. y *Leucocytozoon* spp.) en las muestras.

**Conclusiones**: Sólo el 4.4 % de aves en Río Grande presentaron ectoparásitos, mientras que todas las aves en Maranhão presentaron alta intensidad de ectoparásitos. Durante la migración por Bahía de Delaware en mayo, la proporción de aves con ectoparásitos del norte y sur no fue significativamente diferente, indicando que muchas aves se infectaron durante la parada trófica en la migración norte. La hipótesis de los parásitos predice que los correlimos gordos migrarían hacia zonas marinas de baja prevalencia de parásitos para reducir las posibles consecuencias en el valor adaptativo producida por la alta carga de ectoparásitos en la zona tropical de Maranhão. Sin embargo, también puede existir un aumento de la mortalidad asociada a la migración de larga distancia que se requiere para alcanzar los lugares templado-fríos en Tierra del Fuego. No se detectó presencia de los parásitos sanguíneos (*Plasmodium* spp., *Haemoproteus* spp. y *Leucocytozoon* spp.) en todo el estudio lo cual se asocia con la baja incidencia de vectores en las costas marinas. Las observaciones en las áreas de invernada son consistentes con el papel de la presión parasitaria en la evolución de las estrategias de migración del correlimos gordo. Sin embargo es importante extender los estudios en Bahía de Delaware ya que los resultados sugieren que las aves de Tierra del Fuego podrían estar en desventaja debido al largo vuelo y a la posible adquisición de ectoparásitos en migración o en la bahía, mientras que las aves provenientes de Maranhão sólo sufrirían las altas cargas de ectoparásitos. Es muy importante continuar con el análisis de patógenos y endoparásitos para estudiar el alcance de esta hipótesis en la evolución de las migraciones de las aves playeras.

**Palabras claves**: correlimos gordo, sitios de invernada en Sudamérica, ectoparásitos, parásitos sanguíneos
INTRODUCTION

Costs of migration are usually evaluated in terms of energy expenditure or risk of predation (Alerstam and Lindstrom, 1990; Videler, 1995). However, environmental parasite loads may also modulate migration strategies (Ashford, 1971; Valkiunas, 1993; Piersma, 1997). Parasite prevalence can vary markedly between habitats and host species (Bennett et al., 1992, Mendes et al., 2005). Many birds are hosts to numerous parasite species, either ectoparasites (Clayton, 1991) or endoparasites (Dronen and Badley, 1979; Canaris and Kinsella, 2001, Fallon et al., 2003; Valkiunas et al., 2004; Mendes et al., 2005) of variable pathogenicity. Ectoparasites can affect birds by directly hindering their success in obtaining food and by acting as vector and invertebrate hosts to several microorganisms. For example, lice and mites infest skin and feathers leaving their hosts susceptible to secondary infections (Philips, 1990).

Blood parasites represent a complex spatially heterogeneous host-parasite system having ecological and evolutionary impact on host populations. Three closely related genera, Plasmodium, Haemoproteus and Leucocytozoon are commonly found in wild birds, and infections in highly susceptible species and age classes may result in death (Atkinson, 1999).

Parasitic infections are predicted to additionally play an important role in patterns of habitat selection and spatial behavior, including long-distance migration (Piersma, 1997). Differences in the parasite loads and habitat use between bird species represent basic elements to explain differences in shorebird distribution patterns (Piersma, 1997). As some species of shorebirds have low resistance to parasites and pathogens, Piersma (1997) hypothesized that the evolution of migration in some species of shorebirds could be related to spatial variation in parasite loads at different wintering and breeding sites. Specifically, he suggested that long-distance migrants might trade-off investment in immunocompetence against energetically costly rapid growth of chicks in the Arctic tundra breeding sites and prolonged flights to marine shore wintering sites. Both of these environments are thought to be parasite-poor, and would perhaps explain why long distance migration has evolved in many species of shorebirds.

Long-distance migrant red knots, Calidris canutus rufa (Scolopacidae) show a dichotomy of habitat choice throughout the year (Piersma, 1997). This subspecies breeds in the high Arctic tundra of Canada and spends the non-breeding season in three discrete populations in tropical and cold-temperate southern coastal areas in the Americas (Morrison and Harrington, 1992). A population thought to be of 3,000+ birds winters on the coasts of Maranhão in tropical northern Brazil (Ferreira Rodriguez and Lyra Lopes, 2000; Baker et al., 2005), and another population of about 2,500-4,000 winters in Florida, USA (Niles et al., 2006; Atkinson et al., 2006). A larger population which has now declined to almost 15,000 red knots undertakes one of the longest migrations in the world to spend the winter in Tierra del Fuego (Morrison et al., 2004). The pronounced differences in migratory pathways, climates and habitats used by birds wintering in the tropics of Brazil versus cold-temperate Tierra del Fuego provide an excellent opportunity to test some of the basic assumptions of the parasite hypothesis proposed by Piersma (1997). Therefore, the objective of this study was to estimate the prevalence of ectoparasites and blood parasites in red knots in their both South American wintering areas, at the southern terminus of the flyway in Argentine Tierra del Fuego versus tropical Maranhão in northern Brazil. Parasite distribution is related to different habitats and climate zones, and tropical zones generally have a higher parasite load than cold-temperate ones, so we might expect a higher parasite load in Maranhão compared to Tierra del Fuego. However, the likelihood of parasitic infections in shore-
birds are lower in marine habitats (Piersma 1997) that knots use, and this may mitigate parasite pressure in tropical saline wintering sites. Additionally, at least some knots from the wintering population in Tierra del Fuego migrate through Maranhão en route to the Arctic, and both populations of red knots share the last stopover site in Delaware Bay (USA). To assess the risk of parasitic infections of Tierra del Fuego birds passing through the tropics, comparison was also made with birds captured in Delaware Bay coming from both wintering sites in South America.

**Material and Methods**

The study was conducted at three sites used by red knots, the southernmost wintering area at Rio Grande (54° S, 67.5° W) in Tierra del Fuego, Argentina, the tropical wintering area at Maranhão (0.07° S, 44.8° W), Brazil, and the last major refueling site in Delaware Bay in the USA (39.11° N, 075.14° W) before departure to the Arctic breeding grounds (fig. 1). Birds were captured by cannon net and sampled at the extreme southern wintering site soon after arrival from the southwards migration in Rio Grande in November 2004, during the wintering period in Maranhão in February 2005, and during refueling in Delaware Bay in May 2005. All sampled birds were adults. Catches in Delaware Bay contain a mix of birds from the three wintering populations so only those birds that had the feather stable isotope signatures from the southern population (Tierra del Fuego) and northern populations (Maranhão and Florida) were considered (Atkinson et al., 2006). δ13C and δ15N values were obtained from the sixth primary covert, measured ascendanty, using continuous flow isotope ratio mass spectrometry (CF-IRMS) (Atkinson et al., 2005).

Abundance of ectoparasites was estimated visually on the head, breast, wings and tail following Clayton and Drown (2001). Because of limits on holding times of birds, ectoparasites were classified into abundance categories of 1-100, 101-200, 201-400 and > 400 ectoparasites on each bird. The ranking score protocol is an attempt to cover the variation from no ectoparasites to high infestation, and provides a straightforward approach to reducing processing time for each bird (Dietsch, 2002). Ectoparasites were removed from birds as described in Neves et al., (2000) and preserved in 70% ethanol for later taxonomic identification. Samples from the brachial vein to check for hematoparasites and preserved the blood in ethanol. DNA was isolated from blood in SDS-EDTA.

**Fig. 1.**—Location of study sites: wintering sites at Rio Grande (54° S, 67.5° O), Tierra del Fuego and Maranhão (0, 07° S, 44,8° O). The last stopover site Delaware Bay, (39.11° N, 075.14° W) on the northward migration to the Arctic breeding sites in Canada.

[Ubicación de las localidades de estudio. Áreas de invernada: Rio Grande (54° S, 67,5° O), Tierra del Fuego y Maranhão (0, 07° S, 44,8° O). Última parada trófica Bahía de Delaware (39.11° N, 075.14° W), antes llegar a las áreas de reproducción en Canadá.]
alysis buffer followed by a phenol-chloroform precipitation, and DNA concentration in each sample was checked by running extractions in 1.8% agarose gels. Samples were screened for the presence of avian malaria (*Plasmodium* spp. and *Haemoproteus* spp.) by attempting to amplify a 157 bp section of ribosomal RNA sequence from the 6-kb mitochondrial DNA genome of those parasites (Fallon *et al.*, 2003). No molecular assays were performed to determine the presence of other hematozoan parasites, but instead they were searched for in blood smears. Blood smears were prepared on individual microscope slides, air-dried, fixed with methanol for three minutes and stained with Giemsa. The smears were examined under 400X magnification for extra-erythrocytic blood parasites (i.e. *Trypanosoma*), and under 1,000X magnification with oil immersion for intra-erythrocytic parasites (i.e. *Haemoproteus, Plasmodium, Leucocytozoon*) as in Merino (1999). Each smear was analyzed following the suggestions of Cooper and Anwar (2001).

Tests for analyses of parasite abundance were made using nonparametric statistics (Mann-Whitney U-Test, Kruskal-Wallis, Spearman’s rank correlation coefficient) due to the non-normal distribution (Kolmogorov-Smirnov test) and heteroscedasticity (Levene’s test) of the data (Sokal and Rohlf, 1995). To test for differences in the proportions of birds that were parasitized in different sites, arcsin transformed data were used to calculate the test statistic $t_s$ and doubled the probability in a normal deviate table for a two-tailed test as recommended by Sokal and Rohlf (1995). All statistical analyses were performed using STATISTICA, (data analysis software system, 2001) version 6.

**RESULTS**

Ectoparasites species identified on birds from Maranhão and Delaware Bay were *Actornithophilus umbrinus* (Burmeister, 1838) and *Actornithophilus pediculoides* (Mjöberg, 1910). *Lunaceps incoenis* (Kellogg and Chapman, 1899) was only identified on birds from Delaware Bay. Ectoparasites from Rio Grande could not be identified due to deterioration of the sample caused by a poor quality of the preservation medium.

Only five of the 113 red knots checked in Rio Grande, in Tierra del Fuego, in November 2004 had ectoparasites and they were scored in the abundance category of one to 100 individuals. In contrast, all 36 birds sampled in Maranhão had ectoparasites, and almost 40% were scored in the > 400 category (table 1). Ectoparasite loads in wintering populations were significantly higher in tropical Maranhão (Mann-Whitney U Test, $P < 0.05$) than in cold-temperate Tierra del Fuego (fig. 2).

Of 256 birds captured after they had arrived on the northwards migration in Delaware Bay in May 2005, 174 (68%) had ectoparasites. Using feather isotopes it was possible in the Delaware Bay sample to identify 90 birds from northern tropical sites in Maranhão and Florida (birds that made shorter migrations) and 66 birds from southern cold-temperate sites in Tierra del Fuego (birds that made longer migrations) (Atkinson *et al.* 2006), though it is not possible to clearly distinguish birds from Maranhão and Florida. Although proportions of parasitized birds captured at Delaware Bay from different wintering sites were not significantly different (50.0% from northern sites and 40.1% from southern sites, $t_s = 1.24, P > 0.05$), the northern wintering birds tended to have more ectoparasites (table 1).

All assays for blood parasites were negative, either by a PCR test (Tierra del Fuego = 84, Maranhão = 36 and Delaware Bay = 61) or by examination of blood smears (Tierra del Fuego = 97, Maranhão $n = 16$, Delaware Bay $n = 79$).

**DISCUSSION**

As some species of shorebirds have been postulated to have low resistance to parasites and
pathogens following a late Pleistocene bottleneck and loss of genetic variation (Baker et al., 1994), the evolution of migratory strategies in red knots could at least in part be related to environmental parasite loads. To avoid the high parasite environments in the tropics they could migrate to low parasite environments in the far southern cold-temperate areas or winter in marine tropical areas that have lower parasite abundance (Piersma, 1997). Parasites may impose stronger selection pressures on their hosts in the tropics compared to non-tropical climatic zones because parasite abundance in low latitudes is not reduced by the adverse environmental conditions of winter as in high latitudes (Møller, 1998). Thus the parasite load in Tierra del Fuego should be lower than in the tropical Maranhão or in temperate Delaware Bay. Although the percentage of parasitized birds was consistent with this expectation (Tierra del Fuego = 4.4 %, Maranhão = 100 %, Delaware Bay = 44.9 %), these results suggest that many long distance migrants from Tierra del Fuego either suffered ectoparasite infestations during a stopover period in Maranhão, or they were infested subsequently in the large mixed roosting flocks in Delaware Bay. Because birds from Tierra de Fuego have to invest more resources in long distance flights, they might be immuno-depressed and consequently vulnerable to infestations during stopovers in the tropics, or they simply cannot avoid colonization by ectoparasites in mixed roosting flocks with resident birds (Sychra, 2005). In either case the fitness consequences of higher ectoparasite prevalence in red knots remains to be demonstrated, but experiments on cliff swallows (Hirundo pyrrhonota) and rock doves (Columba livia) showed that high ectoparasite loads reduce annual survival of the hosts (Brown et al., 2008).

### Table 1

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<tr>
<th>Sample sites and year [Localidades de muestreo y año]</th>
<th>Feather isotope signatures [Perfil isotópico de las plumas]</th>
<th>N</th>
<th>Categories of ectoparasite abundance (%) [Categorías de abundancia de ectoparásitos (%)]</th>
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</thead>
<tbody>
<tr>
<td>Rio Grande 2004</td>
<td></td>
<td>113</td>
<td>0  1-100  101-200  201-400  &gt;400</td>
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<tr>
<td>Maranhão 2005</td>
<td></td>
<td>36</td>
<td>0  19.4  27.3  16.6  36.1</td>
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<td>Delaware Bay 2005</td>
<td>Northern sites [Localidades norteñas]</td>
<td>90</td>
<td>50  34.4  12.3  3.3</td>
</tr>
<tr>
<td></td>
<td>Southern sites [Localidades sureñas]</td>
<td>66</td>
<td>59.1  36.4  4.5</td>
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Ardeola 55(2), 2008, 193-202
et al., 1995, Clayton et al., 1999). The much higher prevalence of parasitism and higher loads of ectoparasites in Maranhão wintering birds could imply that they might have lower annual survival relative to Tierra del Fuego wintering birds, but this could possibly be offset by higher mortality of the latter population from the risks of a near doubling of migratory distance to the southernmost terminus in the flyway. However, comparative studies of annual survival of each wintering population remain to be done. Although ectoparasite prevalence in red knots does vary in different years, low prevalence was observed in large catches made annually in Tierra del Fuego from 2000-2008 (unpublished data), including in November 2005 a few weeks after birds arrived on the southern migration. This indicates that the southern wintering birds either shed lice in the cold conditions of the Arctic breeding sites and/or on southern migration and arrival in the subantarctic wintering sites. Conversely, the warm and relatively low humidity marine environments in Maranhão and Florida wintering sites would favor proliferation of ectoparasites, as has been shown in other birds (Carrillo et al., 2007).

The real risks associated with ectoparasites are in the six month exposure period when birds are wintering in tropical Maranhão, consistent with a possible role for parasite pressure and explaining why more birds migrate to Tierra del Fuego than to Maranhão.

Prevalence of blood parasites is very low in migrating shorebirds (Atkinson, 1999; Mendes et al., 2005), and thus it was not surprising that all samples of red knots were negative. The degree of exposure of host species to parasite vectors is one of the main factors explaining parasite prevalence (Clarabuch and González-Solís, 1998). Vectors, pathogens and hosts each survive and reproduce within a range of optimal climatic conditions. Temperature and precipi-
tation are the most important, while sea level, elevation, wind and daylight duration are also important (World Health Organization, 2003). Thus, the apparent absence of blood parasites in some bird taxa is commonly explained by the absence or scarcity of parasite vectors (Bennett et al., 1992; Figuerola, 1999; Jovani et al., 2001). Lack of blood parasites has also been attributed to the good immunological capabilities of the host (Ricklefs, 1992; Forero et al., 1997) or to the lack of a suitable host-parasite assemblage (Blanco et al., 1998). Additionally, Martínez-Abraín et al. (2004) hypothesized that highly mobile ectoparasites might exclude dipteran vectors of blood parasites in bird species with high prevalence and loads of ectoparasites. The absence of blood parasites in red knots is also compatible with the parasite hypothesis of bird migration because the marine habitats they use have a low incidence of suitable vectors such as biting insects, especially in cool climates (Piersma, 1997).

Observations in each South American wintering site could support a role for parasite pressure in the evolution of migration strategies in red knots. However, it will be very important to extend studies to birds captured at Delaware Bay during northward migration, since findings here from birds with northern and southern isotopic signatures do not seem to completely support the hypothesis. Southern populations would have possible detrimental effects of longer migratory distances and ectoparasites load acquired during migration or at the bay, whereas northern populations only would suffer the negative effects of the parasite load. However, it is important in future to extend tests of the parasite hypothesis to other pathogens and endoparasites to help explain the evolution of migratory strategies in shorebirds, and to evaluate immunocompetence levels of birds in each wintering site.

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**Lic. V. D’Amico** is a PhD. student working on aspects of the migration of red knots related to parasites, pathogens and body condition in non-breeding areas in South America. She is actually involved in a BBVA international project related to climate change and conservation of shorebirds in South America. **Dr. Bertellotti** and **Prof. Dr. Baker** are the PhD. supervisors of D’Amico, and their topic is mainly the conservation of avian species. **Dr. Bertellotti** is a CONICET researcher and **Dr. Baker** is the Head of the Department of Natural History in the Royal Ontario Museum, and Professor of Ecology and Evolutionary Biology in the University of Toronto, Canada. **Dr. Tellino Junior** is a Professor in the University of Pernambuco, Brazil and he works on avian ecology, including ectoparasites. **Lic. González** is an avian researcher working on the conservation and migration strategies of red knots. All authors are involved in migrant shorebird conservation.