Red Knots *Calidris canutus rufa* at their farthest south: an international expedition to Tierra del Fuego, Argentina, in February 1995

Allan J. Baker, Rubén E. Manriquez, Luis G. Benegas, Daniel E. Blanco, Oksana Borowik, Eugenio Ferrando, Petra de Goeij, Patricia M. González, Javier González, Clive D.T. Minton, Mark Peck, Theunis Piersma & M. Silvina Ramírez

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As part of a broader research programme aimed at elucidating the ecological, physiological, behavioural and genetic factors shaping the recent evolution of migratory routes in Red Knots, we mounted an international expedition to Tierra del Fuego to study these birds at their southernmost terminus in the world. One large catch of 850 Red Knots was made in front of the town of Río Grande, and 599 birds were processed for morphometrics, body mass, moult, and age. Of these, 396 were colour-banded with orange flags and colour combinations specific to Río Grande. Among the catch were eight retraps, including six birds banded in Brazil, one in the USA, and one unknown. Birds banded at Río Grande were later resighted during migration at five sites on the Atlantic coast of Argentina and in Brazil. Ecological research showed that Red Knots at Río Grande were feeding on *Mytilus edulis* and an abundant bivalve *Darina solenoides* that provided big prey items. Thus feeding conditions seem to be very favourable, and may help explain why Red Knots find it worthwhile to migrate to this relatively cold and energetically costly southern locality in the nonbreeding season. The success of our expedition bodes well for future collaborative studies along the flyway, and opens the door for exciting advances in the fine details of migration, population structure, and conservation of Red Knots.

Calidris canutus en el sur más lejano: una expedición internacional a Tierra del Fuego, Argentina, en febrero de 1995.

Como parte de un programa amplio de investigación dirigido a elucidar los factores ecológicos, fisiológicos, conductuales y genéticos que modulan la evolución reciente de las rutas migratorias de *Calidris canutus*, realizamos una expedición internacional a Tierra del Fuego para estudiar estas aves en el extremo más austral del mundo. Se realizó una gran captura de 850 individuos de *C. canutus* en la ciudad de Río Grande. Se midieron parámetros morfométricos, la masa corporal, la muda y la edad de 599 individuos; 396 de ellos fueron anillados con banderas naranjas y las combinaciones específicas de colores para Río Grande. Se recapturaron 8 individuos, 6 anillados en Brazil, 1 en Estados Unidos y uno de origen desconocido. Los individuos marcados en Río Grande fueron reavistados más tarde durante su migración en tres sitios de la costa Atlántica de Argentina y Brazil. Los resultados de la investigación ecológica, muestran que *C. canutus* en Río Grande estuvo alimentándose de *Mytilus edulis* y de un abundante bivalvo, *Darina solerioides*, ambos proporcionaron grandes itemes de presa. Así las condiciones alimenticias parecen ser muy favorables y podrían explicar porque *C. canutus* encuentra conveniente migrar después de la estación reproductiva a esta localidad austral relativamente helada y costosa en términos energéticos. El éxito de nuestra expedición se vislumbra en los futuros estudios en conjunto a lo largo de las rutas migratorias y en los progresos exicitantes en relación a los detalles finos de la migración, la estructura de las poblaciones y la conservación de *Calidris canutus*.

A.J. Baker, M.K. Peck & O. Borowik, Department of Ornithology, and Centre for Biodiversity and Conservation Biology, Royal Ontario Museum, 100 Queen's Park, Toronto, Ontario, Canada.
R.E. Manriquez & E. Ferrando , Dirección General de Medio Ambiente, Provincia del Tierra del Fuego, San Martin #450, (9410) Ushuaia, Argentina.
L.G. Benegas, Ushuaia 661, (9420) Río Grande, Tierra del Fuego, Argentina.
D.E. Blanco, Wetlands for the Americas, Monroe 2142, (1428) Capital Federal, Argentina.
Petra de Goeij & Theunis Piersma, Netherlands Institute for Sea Research (NIOZ), P.O. Box 59,

1790 AB Den Burg, Texel; and Zoological Laboratory, University of Groningen, The Netherlands.
J. González, Vertebrates Laboratory, Department of Ecological Science, University of Chile, Las Palmeras # 3425, Post-box # 653, Santiago, Chile.
P. González, Dirección de Fauna, Ministerio de Economía, C.C. 84, Pedro Morón 385, (8520) San Antonio Oeste, Río Negro, Argentina.
C.D.T. Minton, Australasian Wader Studies Group, 165 Dalgetty Road, Beaumaris, Victoria 3193, Australia.
M.S. Ramírez, 25 de Mayo 3051, (9420) Río Grande, Tierra del Fuego, Argentina.

INTRODUCTION

Red Knots have long fascinated biologists because they not only breed at such high latitudes across their northern circumpolar range, but also because they undertake long migrations to southern wintering sites (Piersma & Davidson 1995). The southermost terminus of migrant flocks anywhere in the world is in the Argentine and Chilean sectors of Tierra del Fuego, where birds from North America congregate in the relatively cold climate of a subantarctic austral summer.

Recent genetic work on the various subspecies of Red Knots around the world has established that they have very low genetic variability, suggesting that extant populations are derived from a small founder population that barely survived the viscissitudes of the Pleistocene glaciations. This finding carries with it the surprising conclusion that expansion of this remnant population to the present world-wide range of the species occurred very recently, possibly within the last 10,000 years or so (Baker *et al.* 1994). If this scenario is correct then the hemispheric migration patterns that so impress us today are of very recent origin, and thus we have a unique chance to separate historical effects from current ecological, physiological, and life history factors that have shaped the evolution of migratory behaviour in this species.

We therefore decided to mount an international expedition to Tierra del Fuego to catch a large sample of Red Knots to acquire morphometrics, moult scores, extent of breeding plumage, and blood samples for population genetics analysis. By bringing in an international team to work with local biologists we aimed to characterise the wintering sites ecologically, to accelerate efforts to determine which breeding populations these birds come from and the routes of migration they follow, and ultimately to address the vexing question of why these birds go so far south to overwinter. We also wanted to exchange information with local biologists, to share our knowledge of shorebird biology, and to inspire and assist a new group of people in preserving shorebird populations and habitats in this wonderful region of the world.

GENERAL ACCOUNT OF EVENTS

The international team arrived in Río Grande on 16 February 1995, and was met at the airport by local biologists from Ushuaia and Río Grande. Other biologists from Buenos Aires and San Antonio Oeste arrived soon after, and the complete team of 13 people assembled at the Hotel Isla del Mar to discuss plans for fieldwork, check equipment, and to discuss logistical problems. Our biggest relief was that the vital cannon-netting equipment airfreighted from Clive Minton's group in Melbourne, Australia had arrived safely on the same flight. With his vast experience in cannon-netting birds in difficult conditions in different parts of the world, Clive had arranged to bring all his specialised gear by negotiating a special and more affordable freight rate with Aerolíneas Argentinas, for which we are most grateful.

Two key problems remained to be solved in Río Grande, however, problems that threatened the success of the expedition. First, because it is illegal to airfreight the black powder we needed for the cannon charges, we had arranged through local biologists to secure a supply from Ushuaia. When shown the precious local commodity, Clive was instantly suspicous that the distinctly grey looking pellets were blasting grade powder used in seismic surveys for oil exploration. He feared this would burn too fast and expand quickly, thus blowing apart our only three cannons. An innovative solution appeared when we did the rounds of the local fireworks shops, and fashioned our own supply of black powder from the insides of all the rockets we could find. A series of test firings with different amounts of fireworks black powder on the seashore in front of the hotel not only attracted a throng of curious onlookers but also ended with disappointing fizzles. There was nothing else to do but test the blasting grade powder. Despite loading the cartridge with a cautiously low amount of this powder, when it was fired it split the cannon apart like a peeled banana skin. Emergency repairs over the next day in a local welding shop left us with a functional but decidely shorter cannon, but we reckoned it would still work. Another day later when all seemed lost our hard-working local team members finally located a supply of the correct grade of black powder in a gunshop in Ushuaia, and we were finally in business.

Second, we had a major problem with metal band supplies, primarily due to a misunderstanding as to who would supply them. Fortunately, Patricia González from San Antonio Oeste in Río Negro Province had brought her supply of bands with her, as well as plastic strips for colour bands in the yellow, orange and black combination they were using for Argentinian-caught Red Knots. The team enthusiastically manufactured colour bands, sewed together long lengths of hessian sheeting to cover the keeping cages, and did daily reconnoitres of the high water roosts of the Red Knots while the black powder

saga was being played out. We estimated we had enough metal bands for about 200 Red Knots, and could colour-band about double this number if we were lucky enough to catch that many.

Now that we had the means to catch a good sample of Red Knots, we made plans to train inexperienced members of the team in how to select cannon-netting sites, load charges, set the net and cannons, rig the firing gear, and set the jiggling lines needed to coax birds into the safe firing zone away from the immediate vicinity of the net. Our reconnoitres had established that the oceanic beach at Punta Popper (see companion paper for location map) was an excellent site for a preliminary catch. To avoid any undue disturbance to the Red Knots prior to our full-scale catching attempt, we selected an area where smaller flocks of Three-banded Plovers *Charadrius falklandicus* and White-rumped Sandpipers *Calidris fuscicollis* were frequently observed roosting. We decided to try and catch about 50-100 small waders and train the team how to extract them quickly and safely from the net and hold them in keeping cages. We also wanted to band the birds and teach everyone how to take moult scores, morphometrics, and blood samples. On February 19 our first catching attempt was executed successfully, though we were disappointed to catch only 13 birds. However, the team performed splendidly on a cold windy day in their first effort, attesting to the effectiveness of an extensive briefing by Clive, Allan and Theunis prior to the event. We concluded that the team was ready to attempt a larger catch, and that we must try for Red Knots next because the favourable spring tides would not persist much longer.

Because of the high spring tides and continued disturbance by humans and dogs, most of the shorebirds had in the previous few days switched their high tide roost from the sandspit at Punta Popper to the beach in front of the Naval Station in Río Grande. Following another extensive briefing, the team set the cannon net at this site approximately three hours before high water on February 20. About two hours later a good core of Magellanic Oystercatchers formed in front of the net and soon attracted large flocks of Red Knots, Hudsonian Godwits *Limosa haemastica*, and smaller shorebirds. The day was cold and windy, and the birds packed densely together on the roost, making it difficult to accurately judge how many Red Knots were in the firing area. Our goal was to try for a catch of about 300 Red Knots, and the biggest concern was to get birds out of the net and into the keeping cages away from the flood tide as quickly as possible. As firing time approached some unfortunate team members were instructed to strip down to their shorts or underwear (despite the inclement weather) to work at the seaward edge of the net and prevent birds getting wet in any tidal surges. The net was fired, and the team raced down the beach to discover we had an unexpectedly large catch of shorebirds, with about three times as many Red Knots as we had expected. We later realised that many Red Knots were concealed among the flocks of oystercatchers and thus did not figure in our estimates of how many were in front of the net.

Once the net and its catch was safely moved up the beach we made an immediate decision to lift sections containing most of the oystercatchers and release them so we could concentrate on the Red Knots. To increase the numbers of people extracting birds from the net and thus deal efficiently with the large catch, we pressed local bystanders (mostly teenage school children) into service to carry birds to the keeping cages. This not only worked beautifully but also got the community involved and interested in what we were doing.

The team then split into two groups, one banding the birds, and the other measuring, recording moult scores, and taking blood samples. We adopted the method used by Argentinian members of the team of sealing colour bands with screwdriver blades heated in a primus, rather than using glue as done by most other groups. Besides, we welcomed the heat given off by the primus as we huddled against the cold, and some of us could not help but contrast these conditions with the 40 C + temperatures we had experienced banding Red Knots in northwest Australia the previous March! Biting winds, rain and hail forced the teams to work inside covered vehicles provided by the Dirección General de Medio Ambiente and locals, and personnel from the Naval Station kindly erected a tent to provide additional shelter. The hessian sheeting covering the keeping cages served admirably in protecting the birds from the weather and kept them warm throughout the processing period. During the processing operation we were visited by a local TV crew and filmed in action under expert commentary by Rubén Manriquez, and that evening we were greeted all over town by people who had seen us on the news. We also featured prominently in the local newspaper later that week, and all in all enjoyed wonderful PR for our work and for shorebird conservation in Tierra del Fuego.

On subsequent days we cannon-netted small shorebirds on Punta Popper and did field surveys of shorebird populations at Bahía San Sebastián to the north of Río Grande. Although large numbers of Hudsonian Godwits and smaller flocks of Red Knots were found there (see companion paper), no suitable cannon-netting sites could be located on these vast mudflats. By February 24 over half the team departed for home, and the remaining members assisted with further field surveys and with ecological studies of the Red Knots and their wintering sites spearheaded by Theunis Piersma and Petra de Goeij.

CATCHES, COLOUR-MARKING, RECOVERIES AND RESIGHTINGS

Consistent with the objectives of the expedition, the biggest catch we made was the one on February 20 when we caught about 850 Red Knots, 34 Hudsonian Godwits, and about 150 Magellanic Oystercatchers. Of the 850 Red Knots captured, 599 were processed for data on their morphometrics, moult, body weight, and age. Of these birds, 396 were colour-banded with the following combination specific to Tierra del Fuego: right leg, metal from the CENAA (National Banding Centre, Instituto Miguel Lillo, Tucumán, Argentina) on the tibia, black on

the tarsus; left leg, orange flag on the tibia, yellow on the tarsus. Details of the birds banded with metal bands in this catch, and of small waders we caught on other occasions (all of which were banded), are shown in Table 1.

Table 1. Shorebirds banded with metal bands at the WHSRN Hemisphere Reserve 'Costa Atlántica de Tierra del Fuego' near Río Grande in February, 1995.

Species	Numbers	Bands
Calidris canutus	198	XA 16900 - 17000, 17103 - 17199
Calidris fuscicollis	145	YA 12601 - 12611, 12628 - 12761
Calidris alba	1	YA 12627
Limosa haemastica	34	T 10101 - 10134
Charadrius falklandicus	15	YA 12612 - 12626
Total	393	

Among the 599 processed Red Knots we were delighted to record eight retrapped birds, seven of which had metal bands. Six of the latter were banded in Brazil (H 08755, H 08954, H 21934, H 21936, H 16325, H 01066) and one was banded in U.S.A. (802-45388). The birds with Brazilian blue flags were banded at Lagoa do Peixe (31° 0' S, 51° 00' W) between 1985 and 1994 by CEMAVE.

From March-July of 1995, special efforts were made to scan migratory flocks of Red Knots to look for birds banded at Río Grande. The five places along the Atlantic coast of Argentina and Brazil from south to north were Fracaso Beach, Península Valdés, Chubut Province (42° 30' S, 64° 00' W), Bahía de San Antonio, Río Negro Province (40° 45' S, 64° 55' W), Punta Rasa, Buenos Aires Province (36° 22', 56° 45' W), Lagoa de Peixe (31° 10' S, 51° 00' W, Río Grande do Sul State, Brazil), and Tramandai district (ca. 29° S, 150 km north of Lagoa do Peixe). At Fracaso Beach between March 9 and 21 1995, Gustavo Pagnoni and Luis Bala observed seven Red Knots banded at Río Grande, and a Knot banded with colour bands but no flag from another place. On April 20 they also saw two Red Knots banded at Río Grande and another individual carrying a green 1982 or 1983 USA flag. At Bahía de San Antonio between March 27 and April 7 1995, Patricia González observed 14 Red Knots banded at Río Grande and three Red Knots with green USA flags (including one from Delaware, 1985). By April 18 Patricia had inspected 2,600 Red Knots for colour bands. At Punta Rasa Daniel Blanco and Andrea Echazú searched intensively for banded Red Knots on the weekend of April 8-9. They observed one bird banded at Río Grande in a flock of 140 Red Knots. Details of each of these resightings are presented in Table 2.

Table 2. Recoveries and resightings of Red Knots banded at Río Grande during the 1995 northward migration period. Also included where possible is the size of the group in which the observation was made, and the total Red Knots counted or estimated at the location.

Date	Place	Recoveries or Resightings	Group size	Total Red Knots
9 March 1995	Fracaso Beach	1		
10 March 1995	Fracaso Beach	2	(Same Group)	
20 March 1995	Fracaso Beach	2		
21 March 1995	Fracaso Beach	2		
20 April 1995	Fracaso Beach	2		
27 March 1995	Bahia San Antonio	1	23	23
3 April 1995	Bahia San Antonio	3	800	3,000
4 April 1995	Bahia San Antonio	7	300-1,500	8,000
5 April 1995	Bahia San Antonio	2	300	3,500
7 April 1995	Bahia San Antonio	1	1,000	3,000
9 April 1995	Punta Rasa	1	140	780
11 May 1995	Punta Rasa	2	44	
22 April 1995	Lagoa do Peixe	5		
28 April 1995	Lagoa do Peixe	1		
May 1995	Tramandai beaches	6	100-300	6,500

Two birds with Río Grande flags were later recaptured at Lagoa do Peixe in Brazil between April 22-28 by Inês Nascimento from CEMAVE, and four more were resighted there on April 22. In the first half of May, six Red Knots banded at Río Grande were observed by researchers from CEMAVE among flocks of 100-300 birds on the beaches of Tramandai district in Brazil.

Additional PR for the expedition was obtained when interviews with Patricia González about the migration of

Red Knots were reported on local television, newspapers and radio in San Antonio Oeste.

AGE STRUCTURE AND MOULT SCHEDULES

In a sample of 597 fully processed Red Knots, only 20 (3.4%) were aged as juveniles, second calendar year birds. The remaining 96.6% were adult or subadult, the latter category (probably birds in their third calendar year) still being recognizable by distinctively older and lighter primaries, lighter and greener leg coloration and paler breeding plumage feathers compared to the adult category. We encountered 113 subadult birds (18.9% of the total) and 464 (77.7%) adults. If juvenile Red Knots during their first half year of life are as likely to end up in Tierra del Fuego as are subadults a year later, and if subadults stay only a single extra northern winter in the south, then the breeding success of Red Knots in the Canadian Arctic must have been much higher in 1993 than in 1994.

Even as early as 20 February, the adult Red Knots were showing heavy contour feather moult, and most of the birds had already completed over half the full breeding plumage. This means that the *C. c. rufa* is about two months ahead of schedule of Red Knots wintering in tropical west Africa, which show comparable stages of breeding plumage only by mid-April. With an average mass of 126.8 g (and a range of 90 to 160 g), the adults were not very fat when we captured them. However, the individuals with the most advanced breeding plumage were also the heaviest, suggesting that these Red Knots were already starting fuel accumulation. Of the 11 adult Red Knots that accidentally died during the catch, five were males.

Among the 34 Hudsonian Godwits, only one juvenile was found, the remainder of the birds being categorized as adults. The godwits were also in heavy moult and were developing breeding plumage. With body mass values ranging from 212 g to 294 g, these birds had as yet accumulated little fuel stores. All the 145 White-rumped Sandpipers captured on 19 and 22 February were adults! Their moult and breeding plumage scores were similarly advanced as in Red Knots. The findings are therefore quite consistent among all three long-distance migratory, arctic breeding wader species: low numbers of juveniles, and for the adults low body masses, heavy contour feather moult and rather advanced stages of breeding plumage development.

FOOD AND FEEDING ECOLOGY OF RED KNOTS

The wintering area for Red Knots at Río Grande is situated at 54° South, and thus the birds have to contend with a relatively cold and energetically costly environment. Migration to such a southerly locality would be expected to be worthwhile evolutionarily if Tierra del Fuego has something extra to offer, such as high quality food supplies for overwintering birds. To investigate this possibility, Petra de Goeij and Theunis Piersma determined the diet of Red Knots by visual observations of feeding birds, examined droppings for shell fragments, and determined the abundance of prey species.

The diet was found to be composed of two species of molluscs, a rather weak-shelled large bivalve *Darina solenoides* found in intertidal sandflats, and the Common Mussel *Mytilus edulis* found in the banks on the restinga. *Darina solenoides* was taken mostly on falling tides, whereas *Mytilus* was eaten during low water and on rising tides. Although most *Darina* are unavailable to Red Knots because they are buried below bill length in the sediment, nevertheless they provide a dependable food supply due to their high density and large size. Intake rates of *Darina* by the Red Knots were high, and this may explain why many of the birds we captured were in heavy contour feather moult and were developing breeding plumage, despite the relatively cold ambient temperatures they were experiencing in February.

Comparative studies of food abundance at more northerly sites along the flyway are needed to put our observations in proper ecological context, and ultimately to test the hypothesis that the best and most reliable feeding conditions occur fartherest south.

POPULATION GENETIC ANALYSES

One of the major research thrusts of our work on Red Knots is to uncover genetic markers that will allow us to define genetically distinct populations and to reconstruct the very recent intraspecific phylogeography of the species. Because extant populations are apparently derived from a single genetically depauperate population that expanded only in the last 10,000 years or so, it is evident now that we need to screen the nuclear genome for rapidly evolving genetic markers that have evolved in this restricted time frame (Baker *et al.* 1994). At least two classes of markers seem potentially useful in this context. Randomly amplified polymorphic DNAs (RAPDs) appear to distinguish the different subspecies of Red Knots, but the banding patterns on agarose gels are often hard to replicate with Polymerase Chain Reaction (PCR) from run to run. Furthermore, they do not seem to distinguish apparently discrete populations within flyways. In this technique a single primer is used to amplify an anonymous region of DNA between two priming sites. Current work in Allan Baker's laboratory, however, is investigating the utility of a modified technique in which two different primers are used, and the bands they produce are compared to the bands produced by either primer alone. Bands that are unique to PCR reactions using both primers are then isolated from gels and sequenced easily because they have either primer at opposite ends of the amplified region. In fungi these regions have been shown to harbour population-specific markers (Burt *et al.* 1994). The increased resolution afforded by the nucleotide sequences of these bands appears to be worth the extra effort and expense.

A more labour-intensive technique is to isolate highly polymorphic short repeats of two, three, or four

nucleotides called microsatellites. Loci with 10 or more copies of the repeat have been shown to be highly polymorphic, and often provide a good number of population genetic markers (Queller *et al.* 1993). This requires cloning of small pieces of nuclear DNA (300-600 bp), and screening the resultant library with synthetic probes containing particular repeats. Because microsatellites are much less common in birds than in mammals, it is necessary to screen many more clones (ca. 10,000 - 20,000 clones) in the former. Once positive clones have been found, they are grown up and sequenced so that primers can be designed in the regions flanking the repeats. Then the alleles can be amplified with PCR and the genotype at each locus can be determined. Different populations are expected to have different suites of alleles and different allele frequencies. Many alleles occur at low frequencies, and thus it is imperative to assay large samples from each population or else sampling error will be a major confounding factor. Both of these techniques require only small amounts of DNA isolated from blood or growing feather quills. Genetic markers will not replace direct demographic data obtained with banding studies, but the two together will be complementary and will allow us to integrate both contemporary and historical components of the population biology of Red Knots.

FUTURE PLANS AND PROSPECTS

Perhaps the most important benefit that accrued from our expedition to Tierra del Fuego is the confirmation that international collaboration among researchers is absolutely vital for shorebird conservation along hemispheric flyways. Our work would not have been possible without international financing and invaluable local logistical support. Another very important benefit was the excellent public relations that accrued from having an international team working in this remote site, and this helped immeasurably in raising the profile of shorebird and habitat conservation at the local level. This very positive outcome hopefully will be able to translate into financial support for ongoing studies on the flyway, for it is obvious to all of us that we have only begun the task. A receptive audience for future work seems to exist among government agencies, non-governmental organisations, and the general public. A proposal to study Knot migration on the flyway covering the period 1996-2000 has already been drafted, as announced in *Wader Study Group Bulletin* 78.

Following the Western Hemisphere Shorebird Reserve Network meeting in May 1995 in Ottawa, four members of our expedition (Allan Baker, Daniel Blanco, Patricia González, and Silvina Ramírez) paid a very brief visit to Delaware Bay in the United States. Our visit was graciously hosted by Larry Niles of the Delaware Department of Fish and Game, who proposed a twinning of Delaware Bay with sites at Tierra del Fuego and San Antonio Oeste in Argentina. The objective of this scheme is to promote conservation and studies of shorebirds at these sites, and hopefully to assist in conservation work and in financing purchase of computer equipment, binoculars, and spotting scopes for active monitoring studies in Argentina. We hope to have yearly exchanges of personnel between these three sites to increase the scope of our work and to foster international collaboration.

As a final postscript, we must report that a two hour scan of a large flock of approximately 10,000 Red Knots feasting on Horseshoe Crab eggs at Delaware Bay ended on a high note when Patricia González spotted a bird carrying a Brazilian flag and colour combination (banded at Lagoa do Peixe in 1992). We were left with the indelible impression that with considerably more banding, monitoring and genetic analysis of populations along the flyway with a truly international team including colleagues in Brazil and the USA, we now have unparalleled prospects of learning the fine details of knot migration and conservation in the Americas by the turn of the century.

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