**UNIVERSIDADE DO VALE DO RIO DOS SINOS - UNISINOS**
**UNIDADE ACADÊMICA DE PESQUISA E PÓS-GRADUAÇÃO**
**PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA**
**NÍVEL MESTRADO**

**ANTÔNIO COIMBRA DE BRUM**

# USO DE HABITAT E MIGRAÇÃO NORTE DO *Calidris canutus rufa* (MAÇARICO-DE- PAPO-VERMELHO) DO EXTREMO SUL DO BRASIL AO CANADÁ

**São Leopoldo**
**2021**

ANTÔNIO COIMBRA DE BRUM

**USO DE HABITAT E MIGRAÇÃO NORTE DO *Calidris canutus rufa* (MAÇARICO-DE- PAPO-VERMELHO) DO EXTREMO SUL DO BRASIL AO CANADÁ**

Trabalho apresentado como requisito parcial para obtenção do título de Mestre em Biologia, pelo Programa de Pós-Graduação em Biologia da Universidade do Vale do Rio dos Sinos – UNISINOS

Orientadora: Profa. Dra. Maria Virginia Petry

São Leopoldo

2021

B893u Brum, Antônio Coimbra de.
Uso de habitat e migração norte do Calidris canutus rufa (maçarico-de-papo-vermelho) do extremo sul do Brasil ao Canadá / Antônio Coimbra de Brum. – 2021.
95 f. : il. ; 30 cm.

Dissertação (mestrado) – Universidade do Vale do Rio dos Sinos, Programa de Pós-Graduação em Biologia, 2021.
"Orientadora: Profa. Dra. Maria Virginia Petry."

1. Limícolas. 2. Monitoramento. 3. Rota. 4. Ambiente. 5. Área úmida. 6. Lagoas. I. Título.

CDU 573

Dados Internacionais de Catalogação na Publicação (CIP)
(Bibliotecária: Amanda Schuster – CRB 10/2517)

ANTÔNIO COIMBRA DE BRUM

**USO DE HABITAT E MIGRAÇÃO NORTE DO *Calidris canutus rufa* (MAÇARICO-DE- PAPO-VERMELHO) DO EXTREMO SUL DO BRASIL AO CANADÁ**

Trabalho apresentado como requisito parcial para obtenção do título de Mestre em Biologia, pelo Programa de Pós-Graduação em Biologia da Universidade do Vale do Rio dos Sinos – UNISINOS

Aprovado em (dia) (mês) (ano)

BANCA EXAMINADORA

Dra. Maria Virginia Petry – Universidade do Vale do rio dos Sinos (Unisinos)

Dr. Uwe Horst Schulz – Universidade do Vale do Rio dos Sinos (Unisinos)

Dr. Lawrence Joseph Larry – Conserve Wildlife Fundation (CWF) – New Jersey

## AGRADECIMENTOS À CAPES

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

## AGRADECIMENTOS

Agradeço a minha orientadora Dra. Maria Virginia Petry por ter me confiado este trabalho e proporcionado trabalhar com esta ave tão inspiradora e me levado a lugares que eu nunca poderia imaginar. Obrigado pelos conselhos e compreensão, quando as coisas não saíam como desejávamos.

Quero fazer um agradecimento muito especial à Julia Victória Grohmann Finger, minha grande colega do LOAM, que esteve comigo neste projeto desde o início, auxiliando em cada detalhe, pela sua doação incondicional em ajudar, revisar e orientar. Pelo companheirismo em campo e pela viajem ao Panamá e tudo mais que vivemos nesse período e ter deixado a vida sempre mais leve. Obrigado minha grande parceira de campo, por todas as músicas que você cantou para nos alegrar.

Ao Leonardo Dorneles pelo seu auxílio em tentar resolver as questões de estatísticas, que não funcionavam e por todos as dicas e paciência que teve comigo durante todo o tempo. Obrigado pela parceria.

Aos meus queridos colegas do LOAM, pelo auxílio na campanha de captura das aves em 2019 e por estarem sempre disponíveis para os campos do litoral, Vitória, Renata, Camila, Arthur, Luiz, Natacha e Carolina.

Aos colegas laboratoristas Douglas e Marlon, pelo auxílio nas saídas de campo, com os mapas e pela parceria e pelas muitas piadas.

À equipe da Conserve Wildlife Fundation, Dr. Larry Niles, Dr. Joe Smith; Dr. Richard Lathrop e Dra. Stephanie Feigin, pelo suporte e compartilhamento de conhecimentos durante a campanha de captura de Red Knots, em abril de 2019 e pelas conversas que se seguiram. Em especial ao Dr. Larry e Dr. Richard que sempre estiveram disponíveis para auxiliar com seus aprofundados conhecimentos sobre as aves limícolas migratórias. “Thank you, with all my heart!”

Aos canadenses Yann Rochepault e Christophe Badin, que também fizeram parte da equipe americana, pelo auxílio em campo, fotos e pela amizade que nasceu neste campo. “Merci mes amis!”

Um agradecimento especial à Dra. Carmem Fedrizzi, que esteve conosco neste projeto e que sem seus contatos talvez a parceria com a equipe americana não tivesse ocorrido e pela sua participação no campo de captura e compartilhamento de seu conhecimento sobre esta espécie.

Aos professores do PPG da Biologia da Unisinos, com os quais aprendi muito durante todo o curso, em especial ao Dr. Uwe Shulz e Dr. Leonardo Maltchik, pelas valiosas contribuições nas bancas de seminário, lapidando o meu trabalho e ao Dr. Marcelo Zagonel, pelas dicas sobre o ArcGis e a Dra. Cristina Sternet por estar sempre disposta em auxiliar.

Aos membros do Clube de Observadores de Aves de Porto Alegre – COA/POA, que aceitaram apoiar este projeto e viabilizaram os nossos campos e continuidade deles. Em especial ao Roberto Dall´Agnol, Glayson Bencke e Gilberto Mülle pelo auxílio em alguns campos e pela dedicação no monitoramento e leitura de bandeirolas dos maçaricos-de-papo-vermelho.

A equipe do Parque Nacional Lagoa do Peixe, pelo suporte com o alojamento e logística, em especial a Riti, Marcia, Magnus e Lauro.

Por fim e não menos importante à minha família, em especial Edison Kufner, que teve que dividir a mesa do escritório comigo, durante todo o tempo da pandemia e me incentiva a continuar estudando e pela compreensão de minhas paixões, entre elas, a paixão pelas aves.


“Voar, voar, subir, subir
Ir por onde for,
Descer até o céu cair,
ou mudar de cor...”

Biafra.

**RESUMO**

Durante a migração para áreas de reprodução ou de invernada o *Calidris canutus rufa* faz paradas de dias ou semanas em *stopovers* que seleciona ao logo de suas rotas. Entre eles o litoral médio, no extremo sul do Brasil, onde também está localizado o Parque Nacional Lagoa do Peixe. Este local é caracterizado por possuir lagoas litorâneas, áreas úmidas, extensas áreas de dunas, canais de drenagem e amplas praias arenosas, ambiente ideal para aves limícolas. Aqui é apresentado os resultados de dois estudos com esta subespécie. O monitoramento de cinco indivíduos, iniciado em abril de 2019, com a utilização de PinPoint GPS Argos 75, a partir da Lagoa do Peixe, com o objetivo de investigar, rotas migratórias e diferenças nas estratégias de migração. O segundo, através de censos de bandos de Red Knots, entre os anos de 2019 e 2020, com objetivo de analisar o uso do habitat durante os processos migratórios e se variáveis relacionadas ao ambiente justificariam o padrão da presença das aves no habitat. No primeiro trabalho foram encontradas diferenças nas rotas, distâncias percorridas, tempo de deslocamento, velocidade de migração, tempos de paragens e conectividade. As aves migraram pela rota do Brasil Central e Rota Atlântica. Uma delas realizou voo direto de 8.300 km, da Lagoa do Peixe até a Baia de Delaware – USA. Outra parou no Maranhão e uma terceira acessou ambiente desconhecido para a espécie, Baía Santa Rosa – Foz do Rio Amazonas, Brasil. A quarta teve um registro na costa Atlântica de Suriname. A velocidade de migração variou entre 53km/dia a 1230km/dia. Aves com melhor condição corporal percorreram maiores distância em menos tempo, acima de 900 km/dia. Este trabalho evidencia a existência de variação nas estratégias de migração dentro da mesma população e demonstra a importância da conectividade de ambientes fundamentais para a viabilidade da subespécie. No segundo trabalho, foram encontradas diferenças entre o tamanho e distanciamento dos bandos. Na migração norte os bandos são maiores e mantêm maior distância entre sí. Durante a migração norte foi detectada a formação de dois agrupamentos com preferências ambientais distintas. Um grupo na extremidade sul parece ter influência do tamanho da área das lagoas e áreas úmidas e os bandos agrupados na extremidade norte a distância de lagoas, áreas úmidas e drenagens foram importantes para explicar suas presenças. Durante a migração sul foram identificados quatro agrupamentos, no entanto os bandos se distribuíam de forma mais uniforme em toda a área, sendo que as variáveis de maior importância para os agrupamentos dos bandos foram distância das lagoas e áreas urbanas. A distância entre os bandos também desenvolveu um papel complementar. As variáveis distância de lagoas, área de lagoa, distância de drenagens e áreas urbanas, foram as variáveis que parecem melhor responder ao padrão de ordenação das presenças dos bandos no habitat. A distribuição dos bandos está estruturada em áreas que possuem alta similaridade das variáveis estudadas, denotando influência do ambiente nos locais de presença das aves de forma diferente quando está migrando para suas áreas de reprodução ou de invernada. Estudos de uso de habitat são fundamentais para identificar ambientes preferências para o maçarico- de-peito-roxo e que devem ser protegidos.

**Palavras-chave:** Limícolas. Monitoramento. Rota. Ambiente. Área úmida. Lagoas

**ABSTRACT**

During migration to breeding or wintering areas, *Calidris canutus rufa* stops for days or weeks in stopovers that it selects along its routes. Among them is the middle coast, in the extreme south of Brazil, where the Lagoa do Peixe National Park is also located. This place is characterized by having coastal ponds, wetland areas, extensive areas of dunes, stream channels and wide sandy beaches, an ideal environment for shorebirds. Here the results of two studies with this subspecies are presented. The monitoring of five individuals, started in April 2019, using PinPoint GPS Argos 75, from Lagoa do Peixe, with the aim of investigating migration routes and differences in migration strategies. The second, through censuses of flocks of Red Knots, between the years 2019 and 2020, with the objective of analyzing the habitat use during the migratory processes and if variables related to the environment would justify the pattern of the presence of birds in the habitat. In the first work, differences were found in routes, distances traveled, travel time, migration speed, stop times and connectivity. The birds migrated along the route of Central Brazil and the Atlantic Route. One of them performed a direct flight of 8,300 km, from Lagoa do Peixe to Delaware Bay - USA. Another stopped in Maranhão and a third accessed an unknown environment for the species, Baía Santa Rosa – Mouth of Amazonas River, Brazil. The fourth had a record on the Atlantic coast of Suriname. The migration speed ranged from 53 km / day to 1,230 km / day. Birds with better body condition covered greater distance in less time, above 900 km / day. This work highlights the existence of variation in migration strategies within the same population and demonstrates the importance of the connectivity of fundamental environments for the viability of the subspecies. In the second study, differences were found between the size and distance of the flocks. In the northward migration, the flocks are larger and maintain a greater distance between themselves. During the northward migration, the formation of two clusters with different environmental preferences was detected. A group at the southern end seems to be influenced by the size of the area of the ponds and wetlands and the flocks grouped at the northern end at the distance of ponds, wetlands and drainages channels were important to explain their presence. During the southward migration four clusters were identified, however the flocks were distributed more evenly throughout the area, and the most important variables for the flock groups were distance from the ponds and urban areas. The distance between the bands also played a complementary role. The variables distance from ponds, pond area, distance from drainages and urban areas were the variables that seem to better respond to the pattern of ordering the presence of flocks in the habitat. The distribution of the flocks is structured in areas that have a high similarity of the studied variables, denoting the influence of the environment in the places where the birds are present differently when migrating to their breeding or wintering areas. Habitat use studies are essential to identify preferred environments for the purple-breasted curlew that must be protected.

**Key-words:** Shorebird. Monitoring. Flayways. Environment. Wetlands. Pond.

**SUMÁRIO**

# 1 INTRODUÇÃO GERAL

## 1.1 Migração

A migração, definida como um deslocamento sistemático, habitual e sazonal de um grupo de indivíduos, é uma estratégia adotada por diversas espécies. (BAUER; HOYE, 2014). A ocorrência de comportamento migratório em uma espécie está atrelada a pressões seletivas, como disponibilidade de alimento, condições climáticas nas áreas de reprodução e invernada, qualidade do ambiente e competição. (COX, 1985; PIERSMA *et al*.,1996). A estratégia migratória utilizada depende da história evolutiva de cada grupo animal, do tempo e das distâncias de migração, das adaptações fisiológicas relacionadas à reposição de energia durante o percurso migratório, da capacidade de respostas às variáveis ambientais, e.g. condições meteorológicas e controle de navegação. (LUNDBERG, 1988; ALERSTAM *et al*., 2003). A idade, sexo e tamanho dos indivíduos também pode alterar os padrões e até definir a ocorrência de migração. (BUCHAN *et al*., 2020). Estas adaptações reverberam em diferenças nas estratégias de migração adotadas pelas espécies. Nas aves migratórias, por exemplo, existem variações interespecíficas nas distâncias percorridas; algumas aves realizam migrações curtas, da ordem de 200 quilômetros e outras percorrem milhares de quilômetros, como as aves limícolas migratórias. (CUETO *et al*., 2008; LEYER *et al*., 2013; SENNER *et al*., 2015; KENTIE *et al*., 2018).

Dentre os diversos sistemas de migração existentes, destaca-se aqui a “migração neotropical ou austral” e a “migração neártica ou boreal". (HAYNES, 1995). Na migração neotropical, as aves que se reproduzem na porção continental da América do Sul, como aves da ordem Passeriformes, migram para o norte no inverno austral. (CHESSER, 1994; OLIVEIRA *et al.,* 2016). O contrário ocorre na migração neártica: aves que se reproduzem nas regiões frias da América do Norte, como as limícolas, migram na primavera para as regiões mais quentes da América Central e América do Sul. (MORRISON; ROSS, 1989; JAHN *et al*., 2013; OLIVEIRA *et al.,* 2016).

Em razão dos longos deslocamentos realizados, o estudo da migração e delimitação das rotas migratórias utilizadas por aves limícolas é desafiador. (MORRISON *et al*., 1980; PIERSMA *et al*., 1996; HILL; RENFREW, 2019). Por exemplo, apesar das áreas de invernada e reprodução do maçarico-de-papo-vermelho, bem como seus principais *stopover*s, serem conhecidas, pouco se sabe sobre as estratégias de migração adotadas durante a migração, como tempos de paradas, direções, dias de chegadas nos *stopovers* e quais áreas estão sendo conectadas no percurso. Estas incógnitas subsistem para a população migrante da América do Sul, em especial, quando as aves passam pelo território do Brasil. (NILES *et al*., 2008).

### 1.2 Uso de Telemetria No Estudo de Rota Migratória

A telemetria animal, definida como a coleta in situ de dados de posição ou variáveis ambientais em pontos remotos, é uma ferramenta chave no estudo do comportamento e uso de habitat da vida silvestre. Seu uso é amplamente difundido para a identificação de rotas migratórias de vertebrados e invertebrados. (LINDBERG; WALKER, 2007; HASSEL *et al*., 2011; JUSTICIA *et al*., 2018; LÓPEZ *et al*., 2014). Impulsionados pelo desenvolvimento tecnológico nesta área, novos modelos de instrumentos de telemetria e transmissão de dados têm sido desenvolvidos e aperfeiçoados a cada ano, sobretudo com a tecnologia de Sistema de Posicionamento Global (GPS, na sigla em inglês). (LINDBERG e WALKER, 2007; BRIDGE *et al*., 2013). Outra vantagem advém da redução do tamanho dos sensores de posição e baterias. Os rastreadores ganham versões cada vez menores, resolvendo o problema do desconforto para os animais de pequeno porte e baixo peso, como é o caso da maioria das aves. (SCARPIGNATO *et al*., 2016; HILL; RENFREW, 2019).

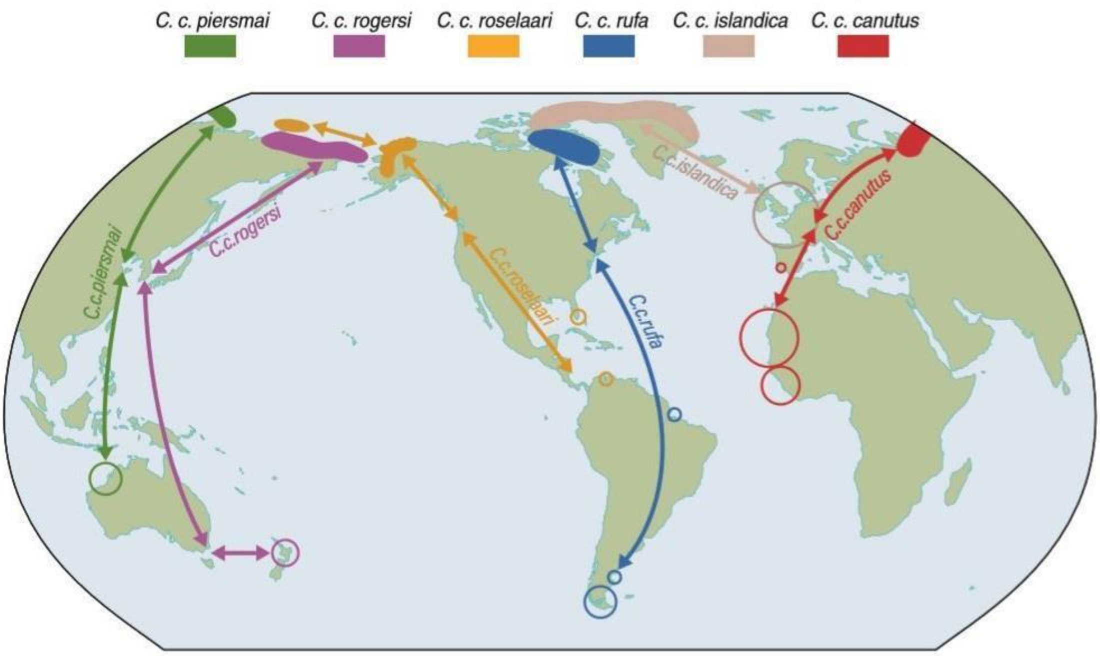
Estes aparatos são ferramentas poderosas para o estudo de rotas migratórias de aves. (WILMERS *et al*., 2015, COOPER *et al*., 2017; SENNER *et al*., 2019). Especialmente os equipamentos rastreadores, tais como geolocalizadores e “PinPoints” que utilizam transmissões via satélite e captura dos dados em tempo real através de programas e aplicativos para computadores e celulares. (ROGERS *et al*., 2010; BRIDGE *et al*., 2013; KERSTUPP *et al*., 2015; SENNER *et al*., 2019). Entre eles destaca-se o modelo PinPoint GPS Argos-75, fabricado pela empresa Lotek, que transmite os dados via satélite utilizando o Sistema Argos. Este é um sistema global de localização e coleta de dados por satélite e tem se destacado no estudo e proteção do meio ambiente no planeta. (SCARPIGNATO *et al*., 2016; ARGOS, 2018). Outro benefício da utilização de equipamento transmissor de dados por satélite é que permite o registro do posicionamento da ave sem necessidade de recaptura para a leitura dos dados. (COOPER *et al*., 2017; SENNER *et al*., 2019).

### 1.3 Espécie Modelo

O maçarico-de-papo-vermelho *Calidris canutus*, pertencente à família Scolopacidae, é uma ave limícola neártica migratória de longa distância. A espécie apresenta seis subespécies que diferem na morfologia, aparência, área reprodutiva, comportamento migratório e distribuição geográfica. (BATTLEY *et al*., 2005; BUHELER, *et al*., 2006; PIERSMA, 2007) (Figura 01). A subespécie *Calidris canutus rufa* reproduz no Ártico canadense e inverna na América do Sul, América Central e algumas regiões na costa atlântica ao sul dos Estados Unidos. A reprodução ocorre entre os meses de junho e julho, principalmente na costa de Nunavut e diversas outras ilhas dispersas na região da Tundra canadense, como a Ilha Southampton, localizada ao Norte da Bahia Hudson. O destino da sua migração de invernada é a Terra do Fogo, no sul da América, sendo que durante o percurso passa pela costa brasileira. (NILES *et al*., 2010; COHEN *et al*., 2009; GILROY *et al*., 2016; LATHROP *et al*., 2018). A população atual desta subespécie é de 42.000 indivíduos e estima-se que população que migra para a América do Sul esteja entre 15.000 a 20.000 indivíduos. (NILES *et al*., 2006; ANDRES *et al*., 2012; SOMENZARI *et al*., 2018). O restante da população inverna na costa de Carolina do Norte; Flórida, Golfo do México e na costa atlântica do Maranhão – Brasil. (BAKER *et al*., 2005; NILES *et al*., 2008; NEWSTEAD *et al*.,2013). Nas últimas décadas a espécie apresentou declínios populacionais intensos, estima-se que houve uma redução de aproximadamente 70% dos indivíduos entre os anos de 1988 até 2012. (ANDRES *et al*., 2012). Atualmente, a espécie é considerada quase ameaçada de extinção (near threatened) pela Lista Vermelha da União Internacional para a Conservação da Natureza (IUCN, na sigla em inglês) (Birdlife International, 2018) e criticamente ameaçado no Livro Vermelho da Fauna brasileira. (ICMBIO, 2016).

A subespécie *C. c. rosselari*, se reproduz no Alasca e migra para a Flórida nos Estados Unidos, podendo chegar eventualmente até o Brasil, na costa maranhense. (BUHELER *et al*., 2006; NILES et al, 2012). O *C. c. islandica* se reproduz na costa do Ártico do Canadá e migra, em grandes bandos, até a Europa. (HASSELL *et al*., 2011). O *C. c pirsma* se reproduz na Nova Ilha da Sibéria e migra até Nova Zelândia e Austrália, passando pelo Mar Amarelo na Ásia. (TOMKOVICH, 2001; BUHELER *et al*., 2006). O *C. c. regorsi* se reproduz no Leste da Rússia e migra para Nova Zelândia e Austrália, também passando pelo Mar Amarelo. (BATTLEY *et al*., 2005; ROGERS *et al*., 2010). O *C. c. canutus* se reproduz no norte da Sibéria e migra até a África, passando pela Europa. (BATTLEY *et al*., 2005).

Figura 01 - Mapa das áreas de reprodução e rotas migratórias das seis subespécies de *Calidris canutus*. As manchas coloridas indicam os locais de reprodução e os círculos vazados as áreas de invernada. As cores correspondem a cada subespécie.




Fonte: mapa com alterações de Buheler; Baker e Piersma (2006).

Anualmente, *C.c.rufa* para completar seu ciclo migratório, chega a percorrer em torno de 30.000 quilômetros, desde suas áreas de reprodução, no norte do Canadá, até as áreas de invernada, no extremo sul da costa da Argentina e Chile. (GONZÁLEZ *et al*., 1996; BAKER *et al*., 2004; NILES *et al*., 2008). Nesse percurso, estudos tem demonstrado que os indivíduos apresentam variação nas suas estratégias, rotas e locais de parada. (BAKER *et al*., 2004; NILES *et al*., 2008; ANDRES *et al*., 2012; ALDABE *et al*., 2015). Variações intrapopulacionais nos parâmetros de migração não são incomuns para diversos grupos de animais. (ALERSTAM *et al*., 2003). Poucos estudos investigaram as rotas de C. c. *rufa* no Brasil, mas sabe-se que a espécie utiliza duas rotas principais, uma pelo interior do país e outra pela costa Atlântica e suas principais áreas de paradas são no estado do Maranhão e no Parque Nacional da Lagoa do Peixe (PARNA). (BAKER *et al*., 2005; NILES *et al*., 2010; OLIVEIRA *et al*., 2016).

O PARNA está localizado na planície costeira do litoral do estado do Rio Grande do Sul. É uma Unidade de Conservação desde 1986 (BRASIL, 1986) e possui reconhecimento internacional, uma vez que integra a Rede Hemisférica de Reservas para Aves Limícolas desde 1991. Esta foi estabelecida na Convenção de Ramsar de 1993, a qual está relacionada à conservação de ambientes aquáticos ao redor do mundo. (ANDRADE, 2003; ICMBIO, 2016). A porção litorânea do Parque é caracterizado por uma longa extensão de praia com larguras que variam entre 50 a 200 metros, propiciando amplo espaço para o forrageio de aves limícolas e costeiras. (LARA-RESENDE, 1987; VOOREN; CHIARADIA, 1990; BENCKE *et al*., 2006; SCHERER; PETRY, 2012). A subespécie *C. c. rufa* utiliza a Lagoa do Peixe e o litoral médio como *stopover* para alimentar-se e para descansar, tanto na migração sul, quando se desloca para as áreas de invernada, quanto na migração norte, quando retorna às áreas reprodutivas. (BELTON, 2000; BENCKE *et al*., 2010; NILES *et al*., 2010; SCHERER; PETRY, 2012).

Estudos da rota migratória desta espécie através de dispositivos de rastreamento remoto de alta definição, a partir do Rio Grande do Sul, ainda não foram realizados. No entanto, é um local fundamental para a viabilidade e conservação da espécie. Deste modo, para que ações de conservação do *C. c. rufa* sejam efetivas, é crucial que as rotas e os locais utilizados como *stopover* sejam bem conhecidos. Da mesma forma é importante entender a conectividade dos ambientes que estas aves estão realizando, uma vez que para completar seus percursos migratórios elas dependem das condições de cada um desses ambientes que acessam e da qualidade das presas que consomem nos locais de parada. (PIERSMA, 2007; BURGER *et al*., 2012). Assim, ter conhecimento de quais locais as aves estão selecionando e conectando ao longo de suas rotas e quanto tempo permanecem neles é fundamental para estabelecer ações que garantam a manutenção da rede de conexões de ambientes essenciais para a espécie cumprir os percursos migratório e obter sucesso na reprodução.

Os habitats utilizados pelos *Calidris canutus* devem cumprir sua função ecológica e biológica, ou seja, ter a disponibilidade de recursos que a ave necessita para, num curto espaço de tempo, repor seus gastos energéticos dos voos de longas distâncias, realizar a muda de penas, descansar e estocar energia suficiente para seguir seu percurso. (ATKINSON *et al*., 2005; NILES *et al*., 2008; PIERSMA, 2007). Portanto, é importante conhecer se o ambiente possui características ambientais que possam justificar a presença das aves, além da distribuição e abundância de presa, durante seus processos migratórios.

Desta forma o estudo de uso de habitat pode prover conhecimentos de quais locais são mais frequentados pela espécie e se existe algum padrão na distribuição espacial nestes locais, fornecendo elementos sobre o comportamento da ave, em especial de como ela está explorando o ambiente, reverberando em orientações que podem embasar ações de conservação.

Para abordar estes tópicos este trabalho foi estruturado em uma introdução geral com objetivos e dois capítulos com metodologia detalhada, resultados, discussão e conclusão. O primeiro se ocupa sobre o estudo das rotas migratórias, estratégias de migração e conectividade de ambientes onde as perguntas que se propõe a responder são: Existem diferenças nas rotas migratórias entre os indivíduos? Quais localidades as aves estão conectando no percurso migratório? Existem diferenças no tempo de deslocamentos, velocidade e distâncias que percorrem entre os locais de paragens?

O segundo sobre o uso do habitat durante o período migratório da subespécie *Calidris canutus rufa*, na migração norte e migração sul e as perguntas que se buscará resposta são: Existe algum padrão na utilização do habitat, locais de presença dos bandos e segregação dos mesmos ao longo da faixa litorânea da área de estudo (Litoral médico do Rio Grande do Sul), durante a migração norte e migração sul de *C. c. rufa*? Estas presenças podem ser justificadas pelas similaridades ou diferenças de características ambientais, tais como distâncias e tamanhos de lagoas frontais, áreas úmidas e áreas urbanas, distâncias de canais de drenagens, largura da praia, bem como variáveis relacionadas aos indivíduos como a distâncias entre os bandos e tamanho dos bandos?

.

# 2. OBJETIVOS

## 2.1 Objetivo Geral

Analisar a rota migratória, tempo de deslocamento e conectividade de ambientes proporcionada pela migração do maçarico-de-papo-vermelho (*Calidris canutus rufa*) desde o Parque Nacional da Lagoa do Peixe, no extremo sul do Brasil, até o Norte do Canadá. Bem como, análise de uso de habitat pela espécie durante sua migração norte e sul, na passagem pelo Litoral Médio do Rio Grande do Sul.

### 2.1.1 Objetivos Específicos

a) Identificar e mapear as rotas de deslocamento de indivíduos da espécie *Calidris canutus rufa* monitorados durante o período de migração Norte;
b) Analisar dados de distâncias percorridas, tempo de deslocamento, velocidade de voo; tempo de parada em *stopover*s, tempo gasto no percurso;
c) Identificar quais ambientes as aves estão conectando durante a sua migração;
d) Inferir se condições físicas dos indivíduos possuem alguma influência no desempenho da ave durante a migração.
e) Analisar se a presença dos bandos ou concentração de bandos em determinadas locais ao longo da faixa litorânea de estudo, durante a migração norte e migração sul de *Calidris canutus rufa*, pode ser influenciada pelas similaridades ou diferenças de características ambientais, tais como distâncias e tamanhos de lagoas frontais, áreas úmidas e áreas urbanas, distâncias de canais de drenagens e largura da praia.
f) Analisar se a organização dos bandos como tamanho e distanciamento é diferente durante a migração norte e migração sul.

**REFERÊNCIAS**


ALDABE, J.; ROCCA, P.; GONZÁLEZ, P.; CABALLERO-SADI, D.; BAKER, A. J. Migration of endangered Red Knots *Calidris canutus rufa* in Uruguay: important sites, phenology, migratory connectivity and a mass mortality event. **Wader Study**, v. 122, n. 3, p. 221-235, 2015.

ALERSTAM, T.; HEDENSTRÖM, A.; ÅKESSON, S. Long-distance migration: evolution and determinants. **Oikos**, v. 103, n. 2, p. 247-260, 2003.

ANDRADE, R.; MARTINI, L. F.; DIEGUEZ, S. P. **Parques Nacionais**: Brasil. São Paulo: Empresa das Artes. 2003.

ANDRES, B. A. ; SMITH P. A. ; MORRISON, G . R. I. ; TREVOR-GRATTO, C. L. Population estimates of North American shorebirds, 2012. **Wader Study Group Bulletin**, v. 119, n. 3, p. 178-194, 2012.

ARGOS, C. L. S. **Argos user's manual**. 2018.

ATKINSON, P. W.; BAKER, A. J.; BEVAN, R. M.; CLARK, N. A.; COLE, K. B.; GONZALEZ, P. M.; ROBINSON, R. A. Unravelling the migration and moult strategies of a long-distance migrant using stable isotopes: Red Knot *Calidris canutus* movements in the Americas. **Ibis**, v. 147, n. 4, p. 738-749, 2005.

BAKER, A. J., GONZÁLEZ, P. M., SERRANO, I. L., JÚNIOR, W. R., EFE, M. A., RICE, S., ... e ECHAVE, M. E. Assessment of the wintering area of red knots in Maranhão, northern Brazil, in February 2005. **Bulletin 107 August**, p. 3, 2005

BAKER, A. J.; GONZALEZ, P. M.; PIERSMA, T.; NILES, L. J.; DE LIMA S. N. I.; ATKINSON, P. W.; AARTS, G. Rapid population decline in red knots: fitness consequences of decreased refuelling rates and late arrival in Delaware Bay. Proceedings of the Royal Society of London. **Series B: Biological Sciences**, v. 271, n. 1541, p. 875-882, 2004.

BATTLEY, P. F.; ROGERS, D. I.; GILS, J. A. V.; PIERSMA, T.; HASSELL, C. J.; BOYLE, A.; HONG-YAN, Y. How do red knots *Calidris canutus* leave Northwest Australia in May and reach the breeding grounds in June? Predictions of *stopover* times, fuelling rates and prey quality in the Yellow Sea. **Journal of Avian Biology**, v. 36, n. 6, p. 494-500, 2005.

BAUER, Silke; HOYE, Bethany J. Migratory animals couple biodiversity and ecosystem functioning worldwide. **Science**, v. 344, n. 6179, 2014.

BELTON, Willian. **Aves do Rio Grande do Sul: Distribuição e biologia**. Universidade do Vale do Rio dos Sinos, São Leopoldo. 98p. 2000.

BENCKE, G. A.; DIAS, R. A.; BUGONI, L.; AGNE, C. E.; FONTANA, C. S.; MAURÍCIO, G. N.; MACHADO, D. B. Revisão e atualização da lista das aves do Rio Grande do Sul, Brasil. Iheringia, **Série Zoologia**, v. 100, n. 4, p. 519-556, 2010.

BENCKE, G. A.; MAURÍCIO, G. N.; DEVELEY, P. F.; & GOERCK, J. M. Áreas Importantes para a Conservação das Aves no Brasil, Parte I–Estados do Domínio da Mata Atlântica. São Paulo: **SAVE Brasil**, 2006.

BRASIL. **Decreto nº 93.546, de 6 de novembro de 1986**. Planalto, 1986. http://www.planalto.gov.br/ccivil_03/decreto/1980-1989/1985-1987/D93546.htm. Acessado em 30 de novembro de 2019.

BRIDGE, E. S.; KELLY, J. F.; CONTINA, A.; GABRIELSON, R. M.; MACCURDY, R. B.; E WINKLER, D. W. Advances in tracking small migratory birds: a technical review of light-level geolocation. **Journal of Field Ornithology**, v. 84, n. 2, p. 121-137, 2013.

BUCHAN, C.; GILROY, J. J.; CATRY, I. e FRANCO, A. M. Fitness consequences of different migratory strategies in partially migratory populations: A multi-taxa meta-analysis. **Journal of Animal Ecology**, v. 89, n. 3, p. 678-690, 2020.

BUEHLER, Deborah M.; BAKER, Allan J.; PIERSMA, Theunis. Reconstructing palaeoflyways of the late Pleistocene and early Holocene Red Knot *Calidris canutus*. **Ardea-Wageningen**-, v. 94, n. 3, p. 485, 2006.

BURGER, J.; NILES, L. J.; PORTER, R. R.; DEY, A. D., KOCH, S.; E GORDON, C. Migration and over-wintering of red knots (Calidris canutus rufa) along the Atlantic coast of the United States**. The Condor**, v. 114, n. 2, p. 302-313, 2012.

CHESSER, R. Terry. Migration in South America: an overview of the austral system. **Bird Conservation International**, v. 4, n. 2-3, p. 91-107, 1994.

COHEN, J. B.; KARPANTY, S. M.; FRASER, J. D.; WATTS, B. D.; TRUITT, B. R. Residence probability and population size of Red Knots during spring *stopover* in the mid-Atlantic region of the United States. **The Journal of Wildlife Management**, v.73, n.6, p.939-945, 2009.

COOPER, N. W.; HALLWORTH, M. T.; MARRA, P. P. Light-level geolocation reveals wintering distribution, migration routes, and primary *stopover* locations of an endangered long-distance migratory songbird. **Journal of Avian Biology**, v. 48, n. 2, p. 209-219, 2017.

COX, G. W. The evolution of avian migration systems between temperate and tropical regions of the New World. **The American Naturalist**, v. 126, n. 4, p. 451-474, 1985.

CUETO, V. R.; LOPEZ DE CASENAVE, J.; MARONE, L. Neotropical austral migrant landbirds: population trends and habitat use in the central Monte desert, Argentina. **The Condor**, v. 110, n. 1, p. 70-79, 2008.

GILROY, J. J.; GILL, J. A.; BUTCHART, S. H.; JONES, V. R.; FRANCO, A. M. Migratory diversity predicts population declines in birds. **Ecology letters**, v. 19, n. 3, p. 308-317, 2016.

GONZÁLEZ, P. M.; PIERSMA, T.; VERKUIL, Y. Food, Feeding, and Refuelling of Red Knots during Northward Migration at San Antonio Oeste, Rio Negro, Argentina

(Alimentacion y Reabastecimiento de Playeros Rojizos *Calidris canutus rufa* Durante su migracion al Norte en San Antonio Oeste, Rio Negro, Argentina). **Journal of Field Ornithology**, p. 575-591, 1996.

HASSELL, C.; SOUTHEY, I.; BOYLE, A.; YANG, H. Y. Red knot *Calidris canutus*: subspecies and migration in the East Asian-Australasian flyway–where do all the red knot go. **Birding Asia**, v. 16, p. 89-93, 2011.

HAYES, F. E. Definitions for migrant birds: What is a Neotropical migrant? **The Auk**, v. 112, n. 2, p. 521-523, 1995.

HILL, J. M.; RENFREW, R. B. Migratory patterns and connectivity of two North American grassland bird species. **Ecology and evolution**, v. 9, n. 1, p. 680-692, 2019.

ICMBIO, 2016. **Relatório anual de rotas e áreas de concentração de aves migratórias no Brasil**. Cabedelo, PB: CEMAVE/ ICMBio, 2016.

JAHN, A. E.; LEVEY, D. J.; CUETO, V. R.; LEDEZMA, J. P.; TUERO, D. T.; FOX, J. W.; MASSON, D. Long-distance bird migration within South America revealed by light-level geolocators. **The Auk**, v. 130, n. 2, p. 223-229, 2013.

JUSTICIA, L. S.; ROSELL, F.; MAYER, M. Performance of GPS units for deployment on semiaquatic animals. **PloS one**, v. 13, n. 12, p. e0207938, 2018.

KENTIE, R.; COULSON, T.; HOOIJMEIJER, J. C.; HOWISON, R. A.; LOONSTRA, A. J.; VERHOEVEN, M. A.; PIERSMA, T. Warming springs and habitat alteration interact to impact timing of breeding and population dynamics in a migratory bird. **Global change biology**, v. 24, n. 11, p. 5292-5303, 2018.

KERSTUPP, A. O.; AYMÁ, G. R.; ROJAS, J. I. G.; VELASCO, A. G. Using Satellite Telemetry to Identify Long-billed Curlew Winter Habitat Use in the Southeastern Corner of the Chihuahuan Desert (Mexico). **The American Midland Naturalist**, v. 174, n. 1, p. 117-132, 2015.

LARA-RESENDE, S.; LEEUWENBERG, F. Ecological studies of Lagoa do Peixe. Final Report to WWF-US, **Washington,** n. 1, p. 52, 1987.

LATHROP, R. G.; NILES, L.; SMITH, P.; PECK, M., DEY, A.; SACATELLI, R.; BOGNAR, J. Mapping and modeling the breeding habitat of the Western Atlantic Red Knot (*Calidris canutus rufa*) at local and regional scales. **The Condor: Ornithological Applications**, v. 120, n. 3, p. 650-665, 2018.

LEYRER, J.; LOK, T.; BRUGGE, M.; SPAANS, B.; SANDERCOCK, B. K.; PIERSMA, T. Mortality within the annual cycle: seasonal survival patterns in Afro-Siberian Red Knots *Calidris canutus canutus*. **Journal of Ornithology**, v. 154, n. 4, p. 933-943, 2013.

LINDBERG, M. S.; WALKER, Johann. Satellite telemetry in avian research and management: sample size considerations. **The Journal of Wildlife Management**, v. 71, n. 3, p. 1002-1009, 2007.

LÓPEZ, L. P.; GARCÍA R.; CLARA, U. V. Individual repeatability in timing and spatial flexibility of migration routes of trans-Saharan migratory raptors. **Current Zoology**, v. 60, n. 5, p. 642-652, 2014.

LUNDBERG, P. The evolution of partial migration in birds. **Trends in ecology & evolution**, v. 3, n. 7, p. 172-175, 1988.

MCKELLAR, A. E.; ROSS, R. K.; MORRISON, R. G.; NILES, L. J.; PORTER, R. R.; BURGER, J.; SMITH, P. A.Shorebird use of western Hudson Bay near the Nelson River during migration, with a focus on the Red Knot. **Wader Study**, v. 122, n. 3, p. 201-211, 2015.

MORRISON, R. I. G.; HARRINGTON, B. A.; LEDDY, L. E. Migration routes and *stopover* areas of North American Red Knot *Calidris canutus* wintering in South America. **Wader Studies Group Bulletin** v.28, p.35-39, 1980.

MORRISON, R. I. G.; ROSS, R. K. **Atlas of neartic shorebirds on the coast of South America**. Ottawa: Canadian Wildlife Service, 1989. 325p.

NEWSTEAD, D. J., NILES, L. J., PORTER, R. R., DEY, A. D., BURGER, J., & FITZSIMMONS, O. N. Geolocation reveals mid-continent migratory routes and Texas wintering areas of Red Knots Calidris canutus rufa. **Wader Study Group Bulletin**, v. 120, n. 1, p. 53-59, 2013.

NILES, L. J.; BURGER, J.; PORTER, R. R.; DEY, A. D.; KOCH, S.; HARRINGTON, B.; BOARMAN, M. Migration pathways, migration speeds and non-breeding areas used by northern hemisphere wintering Red Knots *Calidris canutus* of the subspecies *rufa*. **Wader Study Group Bull**, v. 119, n. 2, p. 195-203, 2012.

NILES, L. J.; BURGER, J.; PORTER, R. R.; DEY, A. D.; MINTON, C. D.; GONZÁLEZ, P. M.; GORDON, C. First results using light level geolocators to track Red Knots in the Western Hemisphere show rapid and long intercontinental flights and new details of migration pathways. **Wader Study Group Bull,** v. 117, n. 2, p. 123-130, 2010.

NILES, L. J.; SITTERS, H. P.; DEY, A. D.; ATKINSON, P. W.; BAKER, A. J.; BENNETT, K. A., ... e SERRANO, I. L. (2008). Status of the Red Knot (*Calidris canutus rufa*) in the Western Hemisphere. In: Status of the Red Knot (*Calidris Canutus Rufa*) in the Western Hemisphere. 2008. p. 1-185

NILES, L.J., H.P; SITTERS, A.D.; DEY, A.J.; BAKER, R.I.G.; MORRISON, K.E.; CLARK, B.A.; HARRINGTON, M.K.; PECK, P.M.; GONZALEZ, P.W.; ATKINSON, K.A.; BENNETT, C.D.T.; MINTON; K.S. KALASZ. Uncertainty about the size and status of Red Knot populations in the Americas is a problem for conservation. **Wader Study Group Bull**, v. 109, p. 39, 2006.

OLIVEIRA, A. D.; BARBOSA, A. E. A.; SOUSA, A. E. B. A.; LUGARINI, C.; LIMA, D. M.; NASCIMENTO, J. L. X.; AMARAL, P. P. Relatório anual de rotas e áreas de concentração de aves migratórias no Brasil. **CEMAVE/ICMBio, Cabedelo**, 2016.

PIERSMA, T.; BRUINZEEL, L.; DRENT, R.; KERSTEN, M.; VAN DER MEER, J.; WIERSMA, P. Variability in basal metabolic rate of a long-distance migrant shorebird (red knot, *Calidris canutus*) reflects shifts in organ sizes. **Physiological Zoology**, v. 69, n. 1, p. 191-217, 1996.

PIERSMA, Theunis. Using the power of comparison to explain habitat use and migration strategies of shorebirds worldwide. **Journal of Ornithology**, v. 148, n. 1, p. 45, 2007.

ROGERS, D. I.; YANG, H. Y.; HASSELL, C. J.; BOYLE, A. N.; ROGERS, K. G.; CHEN, B.; PIERSMA, T. Red Knots (*Calidris canutus piersmai* and *C. c. rogersi)* depend on a small threatened staging area in Bohai Bay, China. **Emu-Austral Ornithology**, v. 110, n. 4, p. 307-315, 2010.

SCARPIGNATO, A. L.; HARRISON, A. L.; NEWSTEAD, D. J.; NILES, L. J.; PORTER, R. R.; VAN DEN TILLAART, M.; MARRA, P. P. Field-testing a new miniaturized GPS-Argos satellite transmitter (3.5 g) on migratory shorebirds. **Wader Study**, v. 123, n. 3, 2016.

SCHERER, A. L.; PETRY, M. V. Seasonal Variation In Shorebird Abundance In The State of Rio Grande Do Sul, Southern Brazil. **The Wilson Journal of Ornithology,** v. 124, p. 40-50, 2012

SENNER, N. R.; VERHOEVEN, M. A.; ABAD-GÓMEZ, J. M.; ALVES, J. A.; HOOIJMEIJER, J. C.; HOWISON, R. A.; STAGER, M. High Migratory Survival and Highly Variable Migratory Behavior in Black-Tailed Godwits. **Frontiers in Ecology and Evolution**, v. 7, p. 96, 2019.

SENNER, N. R.; VERHOEVEN, M. A.; ABAD-GÓMEZ, J. M.; GUTIÉRREZ, J. S.; HOOIJMEIJER, J. C.; KENTIE, R.; PIERSMA, T. WHEN S iberia came to the N etherlands: the response of continental black-tailed godwits to a rare spring weather event. **Journal of Animal Ecology**, v. 84, n. 5, p. 1164-1176, 2015.

SOMENZARI, M.; AMARAL, P. P. D.; CUETO, V. R.; GUARALDO, A. D. C.; JAHN, A. E.; LIMA, D. M.; NASCIMENTO, J. L. X. D. An overview of migratory birds in Brazil. **Papéis Avulsos de Zoologia**, v. 58, 2018.

TOMKOVICH, P. S. A new subspecies of Red Knot *Calidris canutus* from the New Siberian Islands. **Bulletin-British Ornithologists Club**, v. 121, n. 4, p. 257-263, 2001.

VOOREN, C. M.; CHIARADIA, A. Seasonal abundance and behaviour of coastal birds on Cassino Beach, Brazil. **Ornitologia neotropical**, v. 1, n. 1, p. 9-24, 1990.

WILMERS, C. C.; NICKEL, B.; BRYCE, C. M.; SMITH, J. A.; WHEAT, R. E.; YOVOVICH, V. The golden age of bio-logging: how animal-borne sensors are advancing the frontiers of ecology. **Ecology**, v. 96, n. 7, p. 1741-1753, 2015.

# CAPÍTULO I

## NORTHWARD MIGRATION OF *Calidris canutus rufa* AND ENVIRONMENT CONNECTIVITY OF SOUTHERN BRAZIL TO CANADA

(O manuscrito a seguir foi elaborado nas normas de estilo da revista **Wader Study Bolettin** exceto no espaçamento das linhas, justificação do texto e locais de posicionamento das figuras, com o fim de auxiliar na revisão do texto e resultados pela banca examinadora.)

# NORTHWARD MIGRATION OF *Calidris canutus rufa* AND ENVIRONMENT CONNECTIVITY OF SOUTHERN BRAZIL TO CANADA


## ABSTRACT

During northward migration, towards the breeding areas, *Calidris canutus rufa* makes stops of days or weeks in stopovers that it selects along the migratory routes. Among them in the Lagoa do Peixe National Park, extreme south of Brazil. This study presents the monitoring of five individuals of *C. c. rufa*, using PinPoint GPS Argos 75, from Lagoa do Peixe. The objective was to investigate differences in migration strategies. We found differences in routes, distances covered, traveling time, migration speed, stop times and connectivity of environments. Two individuals migrated through the Central Brazil route and two through the Brazilian Atlantic Coast route and another did not migrate. One of the birds made a direct flight of 8,300 km, from Lagoa do Peixe to the Bay of Delaware - USA. Another bird stopped in Maranhão and a third one accessed an unknown environment for the species, Baía Santa Rosa mouth of Amazonas River, Brazil. The fourth bird had only one record in the central- west region of Brazil and another on the Atlantic coast of Suriname. The stopping time of birds in stopovers was 4 to 18 days. The birds made long flights of up to 8,300km, without stops, and short flights, between 1600km to 3,600km, with stops. The migration speed between individuals ranged from 53 km/day to 1230 km/day. Birds with a larger mass (220g) and a longer wing (173cm) covered the longest distance in less time, above 900 km/day. Our study points out important differences in migration strategies, showing the existence of variation within the same population. The selection of a new area, not yet registered for the *C. c. rufa* subspecies reveals the importance of knowing the migration routes and the connectivity of environments essential for the viability of Red Knot.


**Key-words:** Shorebird. Nearctic. Environment. Monitoring. Route.

**RESUMO**

Durante a migração para o Norte, rumo às áreas de reprodução, o *Calidris canutus rufa* faz paradas de dias ou semanas em *stopovers* que seleciona ao logo das rotas migratórias. Entre eles no Parque Nacional Lagoa do Peixe, extremo sul do Brasil. Este estudo apresenta o monitoramento de cinco indivíduos de *C. c. rufa*, com a utilização de PinPoint GPS Argos 75, a partir da Lagoa do Peixe. O objetivo foi investigar diferenças nas estratégias de migração. Encontramos diferenças nas rotas e distâncias percorridas, tempo de deslocamento, velocidade de migração, tempos de paragens e conectividade de ambientes. Dois indivíduos migraram pela rota do Brasil Central e dois pela rota da costa Atlântica brasileira e um não migrou. Uma das aves realizou voo direto de 8.300km, da Lagoa do Peixe até a Baia de Delaware – USA. Outra ave parou no Maranhão e uma terceira acessou ambiente desconhecido para a espécie na Foz do Rio Amazonas, Baía Santa Rosa, Brasil. A quarta ave teve um registro na região centro-oeste do Brasil e outro na costa Atlântica de Suriname. O tempo de parada das aves nos *stopovers* foi de 4 a 18 dias. As aves realizaram longos voos de até 8.300km, sem paradas, e voos curtos, entre 1.600km a 3.600km, com paradas. A velocidade de migração entre os indivíduos variou entre os 53km/dia a 1230km/dia. Aves com massa maior (220g) e asa mais cumprida (173cm) percorreram maiores distância em menos tempo, acima de 900km/dia. Nosso estudo aponta diferenças importantes nas estratégias de migração, evidenciando a existência de variação dentro da mesma população. A seleção de nova área, ainda não registrado para o *C. c. rufa* revela a importância de se conhecer as conectividades de ambientes fundamentais para a viabilidade do Maçarico-de-papo-vermelho.

**Palavras-chave:** Ave Limícola. Neártico. Ambiente. Monitoramento. Rota.

## INTRODUCTION

The Red Knot *Calidris canutus* (Scolopacidae) is a cosmopolitan shorebird which is able to migrate long distances. The subspecies Red Knot *Calidris canutus rufa* reproduces in the Canadian Arctic Archipelago and migrates to the Southern hemisphere in order to winter in Tierra del Fuego in the south of South America (Niles *et al*. 2010, Hassel *et al*. 2011). It is estimated a *C. c. rufa* population worldwide to be around 42,000 specimens, from the whole population is believed that 15,000 to 20,000 individuals migrate until the southern of South America (Niles *et al*. 2010, Andres *et al*. 2012, Somenzari *et al*. 2018). The *C. c. rufa* has suffered a drastic reduction of its population in the past decades (Niles *et al*. 2008, Andres *et al*. 2012, Burger *et al*. 2012). In a study of the wintering population in Tierra del Fuego has shown a drop of 75% between the years of 1986 - 2011 (Morrison & Ross, 1989, Andres *et al*. 2012, Aldabe *et al*. 2015). Census held, in the Delaware Bay, a key stopover to the species in the north hemisphere, between 1986 – 2014, also revealed similar declines (70%) (Andres *et al*. 2012). A census held in Lagoa do Peixe and mid-littoral Rio Grande do Sul coast in 1986, spotted 22,000 birds on a single April day (Harrington *et al*. 1986), In April 2020 we conducted a census on the same areas and we spotted on a single day only 2.800 birds ‘unpubl.data’. The main reasons towards the decline were the drastic reduction on the horseshoe crab *Limulus polyphemus* population in the Delaware Bay, which the eggs are the primary food resource to the Red knots in this stopover, and the destruction of habitat along its migration routes, caused by anthropization, and climate changes (Baker *et al*. 2004, Cohen *et al*. 2009, Niles *et al*. 2010, Burge *et al*. 2014).

Despite of the *C. c. rufa* declines, its IUCN status is listed as Least Concern (Birdlife 2021). In Canada (RLRQ, cE-12.01) and USA the species is considered to be endangered and threatened respectively (US Endangered Species Act in 2014). In Argentina, the species is listed as Endangered ‘Resolution No. 34/2010 of the SESD’, In Brazil is listed as Critically Endangered ‘MMA 2018, Red Book’, which only reflects the declines of the subspecies that is occurring in those countries.

During the migratory movements, both in southward migration and northward migration, the individuals can range about 15,000 kilometers, in each route the birds will stop only in certain sites to rest and feed, the so named stopovers. The Delaware

Bay is one of the most prominent and studied stopover of the subspecies (Harringnton *et al*. 1988, 2010, Niles *et al*. 2008, Burger *et al*. 2012). The arrival of the Red Knots to the bay occur in the late April and early May, overlapping with the horseshoe crab spawning. Its eggs have the necessary amount of nutritional value which will give the birds a chance to restore their energy that was once spent during their prolonged previous migratory flight, that way obtaining the enough body mass required, so they can have the necessary energy to make the journey to the reproduction sites (Baker *et al*. 2004; Morrison *et al*. 2007; Niles *et al*. 2009). However, it's crucial for the individuals to arrive in Delaware Bay good condition, in order to ensure reproductive success (Burger *et al*. 2018). If the individual arrive without the body mass required, it will need to remain longer feeding in the stopovers, this fact can easily delay the migration and may compromise the reproduction (Atkinson *et al*. 2005). In this regard, the body mass obtained in the previous stopovers are fundamental (Burger *et al*. 2018).

The Lagoa do Peixe and the Brazil's southern cost, in particular the mid-littoral coast in Rio Grande do Sul state, are stopovers used by the *C. c. rufa*, mainly during the Northward migration (late March and early April), but also in the Southward migration (September and October) (Harrington *et al*. 1988, Niles *et al*. 2008, Scherer & Petry 2012). Some individuals on the other hand may remain in Lagoa do Peixe during spring days, as seasonal residents, those are in the majority young individuals or birds which don't migrate, for reasons not yet understood (Lara-Resende & Leeuwenberg 1987, Antas & Nascimento 1996, Martínez- Curci *et al*. 2020). Once that Lagoa do Peixe and Rio Grande do Sul coast are one of the last stops before the Delaware Bay, those stopover have been established as strategic areas for the *C. c. rufa* viability, and also other bird species which migrate trans-equatorial and use the region as well (Oliveira *et al*. 2016).

Despite the fact that there are a lot of studies that investigated the migration routes of the *C. c. rufa*, in the EUA's Atlantic coast, Central America (Burger *et al*. 2012, Niles *et al*. 2012, Newstead *et al*. 2013, Mckellar *et al*. 2015), and the migration route in South America is still not well investigated. One Project using geolocation tracking starting in Delaware Bay, identified that birds move both through Central Brazil and the Brazilian Atlantic coast during southward migration (Niles *et al*. 2010). Nevertheless, there is a lack of studies investigating the northward migration and the stopovers that the birds are connecting during their migration journey towards the reproduction sites, mainly with marked birds in the south of Brazil. The present study aims to investigate the *C. c. rufa* migration routes starting in the southern of Brazil, through tracking with GPS devices attached to the Argos system. The travel time and the distances covered will be analyzed, stopovers identified and the time spent on them, the areas connected during these routes and the physical condition of the individuals influenced the migratory parameters inferred. It is expected that there are differences in the migration parameters between individuals, birds with greater mass and wing length will demonstrate a better performance than those with fewer mass and wing length.

## METHODOLOGY

### *Study site*

Red knots were tagged in the Lagoa do Peixe National Park (31°00’46”S, 50°46’31”W; 31°29’00”S, 51°09’51”W), which covers a brackish water lagoon 35 km long and 2 km wide, having a direct connection to the sea through a chanel, as well as sandy beaches, sandbanks, restingas forests and wetlands. The park covers 34,000 ha if the coastal plain of the Rio Grande do Sul state, in the southern of Brazil (Fig. 1), and it is located between the municipal areas of Tavares and Mostardas (Oliveira *et al*. 2016). Since 1986, it has been classified as a Full Protection Conservation Unit (Brasil 1986) and due to its importance for migratory shorebirds, it is internationally recognized as a Biosphere Reserve Site by the Ramsar Convention, to which Brazil is a signatory. The Migratory Wader’s Action Plan ‘PAN Waders’ also recognizes the importance of Lagoa do Peixe for hemispheric birds and highlights it as a stopover for migratory (Icmbio 2016).

Due to the lagoon connection with the sea, the sea waters go in the lagoon, creating marshes which provide for a high biological productivity (Andrade *et al*. 2003), which attracts birds, mainly migratory ones (Frazier, 1996; Bencke *et al*. 2006). The eastern part of the park faces the sea and its beaches are used as a foraging area by visiting birds. The birds investigated in this study were captured in this coastal portion (31°17'25.16"S, 50°57'15.03"W) (Fig. 1).


**Fig. 1** – Study Area: Lagoa do Peixe National Park. The circle indicates the limits of the Park and where the birds were captured.

***Capture and tagging***

As an outcome of another study, individuals of *C. c. rufa* were captured by a cannon net on April 24th, 2019, in a campaign that was carried out during the period of Northward migration of the species. After being captured, the birds were immediately removed from the net and placed in a keeping cage in order to be transported to a containment tunnel. The tunnel was composed of jute fabric and supported by metal arches stuck in the sand (Fig. 2). Once in this tunnel, the bird's feet were in contact with the sand, a substrate that they can recognize and feel safe. The tunnel was also covered by shading, to avoid overheating. Five of the 51 individuals captured were selected for the coupling GPS devices based on their plumage and mass. Only birds with reproductive plumage (chest and red-brown face and gray coverts feathered with black and reddish dots) were selected (Andres *et al*. 2012) and body mass > 200 g. The reasons behind the body mass threshold was made taking into account the fact that this is considered the ideal body mass for an individual to be able to continue the migration (Piersma *et al*. 2005, Aldabe *et al*. 2015, Chan *et al*. 2016) and that the weight of the device that would be fixed on the birds could not weigh more than 3% of the bird's body mass (Cooper *et al*. 2017). A body mass over 200 g is considered to be ideal to an individual begin its migration (Piersma *et al*. 2005). Besides the body mass, it was also taken into account the total wing length, measured from the tip of the primary patch p10 to the wing, using precision calipers.

Fig. 2 – A containment tunnel for the birds

***Tracking***

PinPoint GPS Argos 75 (Lotek) tracking devices weighing 3.7 g were attached to the back of the birds, the GPS were settled 1 cm away from the uropygial gland. The feathers needed to be cut off, in order to place the devices which were glued with a high adhesion, long-lasting cirurgical glue (Cohen *et al.* 2009, Lathrop *et al.* 2018) (Fig. 3a). The devices had a five-centimeter antenna in order to receive the GPS signals, and another eighteen-centimeter antenna for the Argos system (Fig. 3b). The PinPoint tags provide GPS positions acquired at scheduled intervals that are compressed and transmitted via the Argos system. Tags have a limited number of positions that can be captured due to its battery capacity. On 23 Apr the tags were programmed to send record 26 locations that would be collected from 25 Apr 2019 and 4 Oct 2019, resulting in a 161 days period (Table 1), which is the PinPoints battery life (Argos 2018). All data transmitted included time stamps and satellite ID. The GPS position data was transmitted via the ARGOS satellite system and it was processed using Lotek software.

***Ringing***

Each of the individuals received an aluminum ring, following methods in the Brazilian ringing manual and a blue leg flag marked with white inscriptions on (Fig. 3a). The marking process followed the CEMAVA ringing protocol and the international flag marking regulation expected by the Pan Americano for seashore birds, joined by ICMBIO/CEMAVE (Icmbio 2020).

### *Molecular sexing*

It was mandatory to use PCR molecular sexing techniques to determine sex, because the species does not show a clear sexual dimorphism. In order to molecular sex the birds, before fixing the PinPoints, blood was collected by puncture in the ulnar vein, using a sterilized needle. The collected blood was added on a filter paper to sexing cards and sent to a molecular sexing at the Molecular Biology Laboratory of the Vale do Rio dos Sinos University. Primers P2 and P8 were utilized, following the procedures of DNA extraction, amplification ‘PCR’ of a specific segment and electrophoresis in agarose (Gaunt *et al*. 1999).

| Initial Date | Final Date | Interval | Nº Positions |
|---|---|---|---|
| 26/04/2019 | 18/05/2019 | 2 days | 11 |
| 20/05/2019 | 31/05/2019 | 5 days | 2 |
| 01/06/2019 | 14/06/2019 | 3 days | 4 |
| 02/08/2019 | 06/08/2019 | 2 days | 1 |
| 15/07/2019 | 20/08/2019 | 10 days | 2 |
| 10/09/2019 | 25/09/2019 | 8 days | 2 |

**Table 1** – Schedule of the PinPoint GPS Argos 75 schedule. Initial date = starting date of positioning data collection, final date = last date for positioning data collection, interval = interval of days recording the bird’s location, positions = number of positions fix configured for sending location.

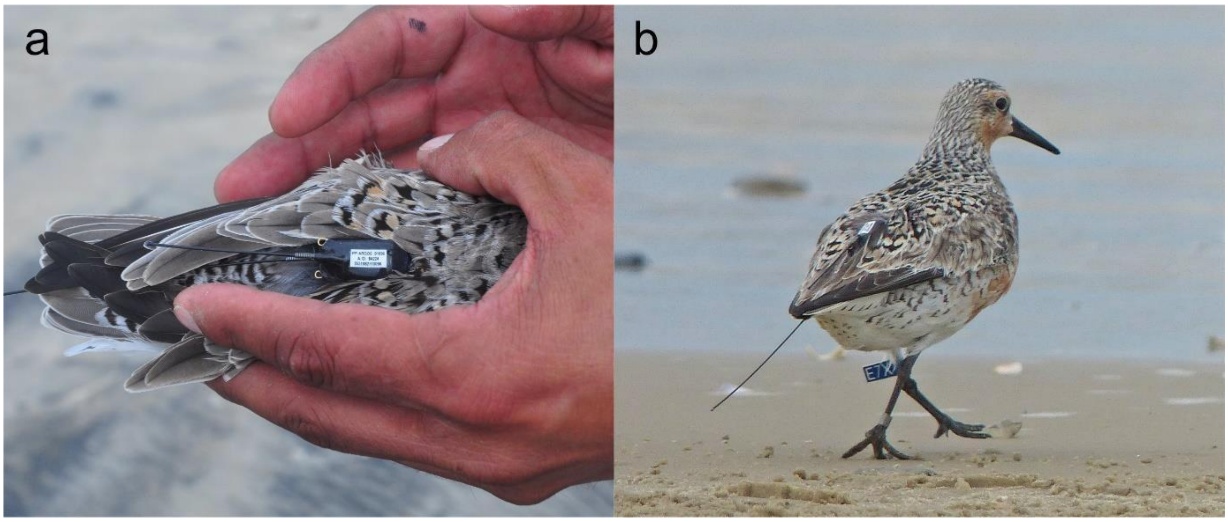



**Fig. 3** - a) Red Knots *Calidris canutus rufa* marked with a PinPoint GPS Argos 75 device and a Brazilian. b) PinPoint GPS device attached to the back near the uropygium.

***Analysis procedures***

Held with the aim to identify whether there were differences in the migratory parameters and migration strategies adopted by the tagged individuals, the total distances covered 'from the place where the birds were marked to the last location recorded by PinPoint' was calculated, and the relative distances 'distance between one position and the next position' for each individual (km). We also calculate the traveling time (days), migration speed (Km/day), average flight speed (Km/h) and time (days) spent on the stopover. The travel time of each bird was calculated by the number of days between the monitoring start date (April 26, 2019) until the date of the first location registered at a stop or stopover. The migration speed was calculated considering the relative distances divided by the number of days between one location and another. The average flight speed was calculated considering the distance between one location and the next, in a straight line, divided by the number of hours it took the bird to travel that distance, making sure that the bird was not stationary between the measured locations. The dwelling or stopping time was determined between the dates of the first position recorded at the stop or stopover location until the date of the last position recorded at the location. The distance measurements were plotted using the measurement tools of the ArcGis 3.4 software (ESRI, Redlands, USA). Due to the low number of individuals sampled (n = 5) and the obtained fixes (n = 14), the individual migration parameters and the relationship of the morphometric measurements with these parameters were analyzed descriptively.

This study was approved by the Ethics Committee for the Use of Animals of the University of Vale do Rio dos Sinos - UNISINOS, according to CEUA No. 05.2018 and was approved by Sisbio No. 60387-7.

## RESULTS

Of the five individuals monitored, four of them migrated and only one (E8A) remained in the Lagoa do Peixe National Park. Only one of those who migrated (E7X) was able to monitor until their arrival in the reproductive areas. All birds were female (see supplementary material S1 for the results of sexing tests), with complete alternating plumage, reproductive plumage. The females displayed a variation in the parameters and migratory routes taken (Table 2 & Fig. 4). Two females (E7X - E7N) migrated via the Central Brazil route (E9Y - V53) and the other two via the Brazilian Atlantic Coastal route (Fig. 4).

The bird E7X made a non-stop flight from Lagoa do Peixe to the Delaware Bay (USA), covering a distance of 8,289 km, flying over the Pantanal region, mid-west Brazil, the Amazon forest and the Caribbean Sea (Fig. 4 & 5). The relative distance ranged, from 1,206 km to 4,916 km, and the migration speed, from 105 km/d to 1,229 km/d, with the maximum measured flight speed being 51 km/h. Restricted to the data generated by PinPoint the journey time of this female bird until its arrival in Delaware Bay was 14 days and the time spent in this stopover would have been at least six days, before leaving towards James Bay – in Canada, flying more 1,658 km, in 16 days. It was not possible to plot the dwell time at this stopover, since we obtained only one registered position fix. From James Bay, the bird travels another 1,206 km, in nine days, to Southampton Island, in Nunavut – Canada, breeding area, where the last fix was registered on 10 June. The total distance covered by this female was 11,170 km, during a period of 45 days.

The bird (E7N) was monitored only in Brazil. She did the same route as the E7X bird passing through the Pantanal, where it had a registered fix coincident with that of the previous bird. This female, after traveling 3,280 km in six days, makes two stops: one near the Xingu River and the other at Caxiuna Bay, state of Pará (Fig. 6b). There is no record for the species in these places. The bird remained in the region for four days before traveling another 614 km, arriving in the Maranhão state, on 8 May, where it stayed for another 14 days. Relative distances ranged from 614 km to 1,646 km and the migration speed ranged from 307 km/day to 817 km/day. The flight speed was 34 km/hr and the total distance covered by this female was 4,050 km, during a period of 12 days.

The V53 bird was also monitored only in Brazil. This female migrated along the Brazilian coastal, making only small flights until making a long flight of 2,028 km, in two days, arriving at the coast of the state of Bahia, northeast Brazil, from where she makes another flight of 1,619 km, in two days, accessing a new area, not yet known for the specie, located in the state of Pará, Santa Rosa Bay, in the Amazon River, where the arrival position fix was registered on 6 May (Fig. 6a). The bird remains in this place for at least four days and there is no more fixing from there. The relative distances ranged from 214 km to 2,028 km and the migration speed between 54 km/day to 1,014 km/day. The maximum speed measured was 42 km/h and the total distance covered was 3,873 km, during a period of eight days.

The E9Y bird had only two registered fixes, one in southwestern Brazil and another one and last on the Atlantic coast of Suriname, already beginning ocean crossing on the way to Delaware. The relative distance was 3,454 km, with a migration speed of 345 km/days. The bird covered a total distance of 4,524 km, during a period of 16 days. The differences in the distances covered and travel times for all females are shown in the graph (Fig. 7).

The monitoring birds established connectivity between Lagoa do Peixe and Delaware Bay (USA); Coast of Maranhão (Brazil); Caxiuana Bay, interior of the State of Pará and Santa Rosa Bay - mouth Amazonas River - coast of Pará with the boundary of the State of Amapá (Brazil) (Fig. 5b & 6a, b).

***Birds conditioning and migratory performance***

The individuals body mass varied between 208-233 g and the wing length between 167-173 cm. Despite the impossibility of statistical data, there seems to be a tendency for birds with greater mass and greater wing length to perform better in migration than birds with less mass and shorter wings (Table 3). Comparing the relative distances between the individuals, we could observe that the bird E7X (mass of 223 g and wings of 173 cm) covered a distance of 6,599 km, in ten days, from Pantanal - MT, where it had its first registered fix, until Delaware, while the bird E7N (mass 208g and wings with 168 cm), it took the bird six days to cover a distance of 3,280 km and made two stops, before moving to Maranhão. When comparing the highest migration speed measured for each individual, the E7X has a better performance (1,229 km/day) while the E7N (817 km/day). Comparing the E7N bird with the V53, which had the best conditioning (mass 233 g and wings 173 cm), it performed better and moved faster, covering a distance of 3,647 km, in four days, reaching a migration speed of 1,014 km/day. However speaking about the E7X bird, its performance is lower, in addition to stopping. It was not possible to establish a relationship between the migration parameters with the bird E9Y, since this bird had only two positions recorded and it is not certain that it stopped somewhere before starting the equatorial crossing.

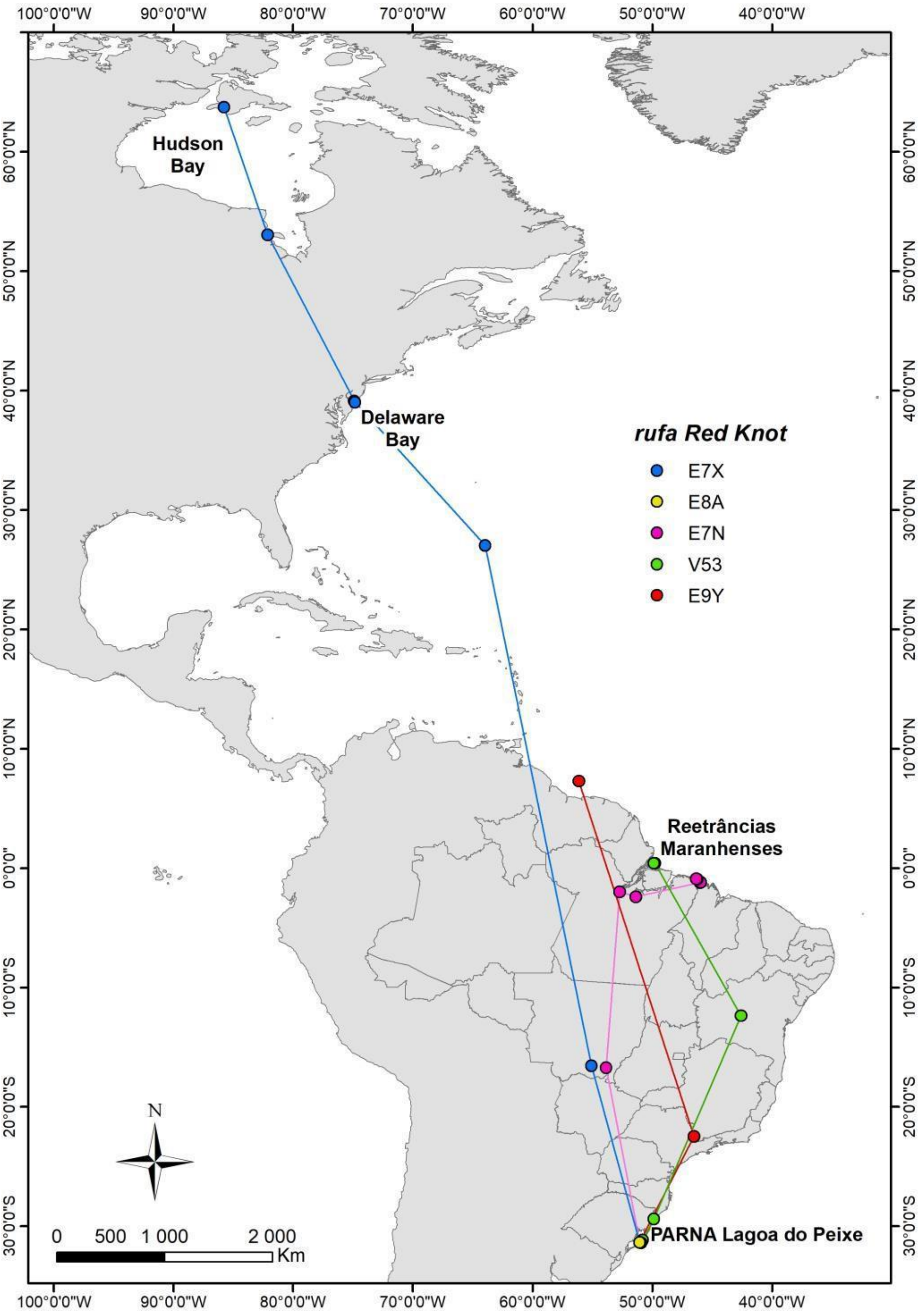



**Fig. 4** - Migratory route used by *Calidris canutus rufa* from the Lagoa do Peixe National Park - Brazil. The route of each bird are marked with different colors and the points are the recorded locations.

| Bird | Date | | Position | | nf | d | rd | ms(Km/d) | fs(Km/h) | Location |
|---|---|---|---|---|---|---|---|---|---|---|
| | Initial | Final | Initial | Final | | | | | | |
| E7X | Apr,30 | May,04 | -16º55 S<br>-55º08 O | 27º03 N<br>-63,93 O | 2 | 4 | 4.916 | 1.229 | 51 | Mato Grosso /Bahamas |
| | May,04 | May,10 | 27º03 N<br>-63,93 O | 39º33 N<br>-74º88 O | 2 | 6 | 1.683 | 280 | - | Bahamas/ Delaware Bay |
| | May,10 | May,16 | 39º33 N<br>-74º88 O | 39º01 N<br>-74º83 O | 3 | 6 | - | - | - | Delaware Bay |
| | May,16 | Jun,01 | 39º01 N<br>-74º83 O | 53º 06 N<br>-82º13 O | 1 | 16 | 1.685 | 105 | - | Delaware/ James Bay |
| | Jun,01 | Jun,10 | 53º06 N<br>-82º13 O | 63º72 N<br>-85º75 O | 1 | 9 | 1.206 | 134 | - | James Bay / Southampton Is. |
| E7N | Apr,26 | Apr,30 | -31º35 S<br>-51º05 O | -16º71 S<br>-53º85 O | 2 | 4 | 1.646 | 411 | - | Lagoa do Peixe/Mato Grosso |
| | Apr,30 | May,02 | -16º71 S<br>-53º85 O | -1º98 S<br>-52º72 O | 1 | 2 | 1.634 | 817 | 34 | Mato Grosso/Pará |
| | May,02 | May,06 | -1º98 S<br>-52º72 O | -2º39 S<br>-51º38 O | 1 | 4 | - | - | - | Pará |
| | May,06 | May,08 | -2º39 S<br>-51º38 O | -1º21 S<br>-52º72 O | 1 | 2 | 614 | 307 | - | Pará/Maranhão |
| | May,08 | May,20 | -1º21 S<br>-52º72 O | -0º89 S<br>-46º32 O | 3 | 12 | - | - | - | Maranhão |
| V53 | Apr,26 | Apr,30 | -31º15 S<br>-50º79 O | -29º39 S<br>-49º85 O | 2 | 4 | 214 | 53 | - | Tavares/Lagoa Itapeva |
| | Apr,30 | May,02 | -29º39 S<br>-49º85 O | -12º38 S<br>-42º59 O | 1 | 2 | 2.028 | 1014 | 42 | Lagoa Itapeva/Bahia |
| | May, 02 | May,04 | -12º38 S<br>-42º59 O | 0º39 N<br>-49º76 O | 1 | 2 | 1.619 | 809 | - | Bahia/Baía Santa Rosa -Amapá |
| | May,04 | May,06 | 0º39 N<br>-49º76 O | 0º39 N<br>-49º86 O | 1 | 2 | - | - | - | Baía Santa Rosa-Amapá |
| E9Y | May,02 | May,12 | -22º48 S<br>-46º51 O | 7º27 N<br>-56º13 O | 2 | 10 | 3.454 | 345 | - | Sertãozinho-São Paulo/ Suriname |
| E8A | Apr,26 | Jul,23 | -31º35 S<br>-51º04 O | -31º35 S<br>-51º04 O | 20 | 90 | - | | - | Lagoa do Peixe |

**Table 2** – The main positions fixes of birds recorded by the equipment. Distances and times between fixes, flight speed, number of fix for each bird and locations for recording the position. d = days; nf = number of fix; rd= Relative distances (km); ms = Migration speed (km/d) fs = flight speed (km/hr).

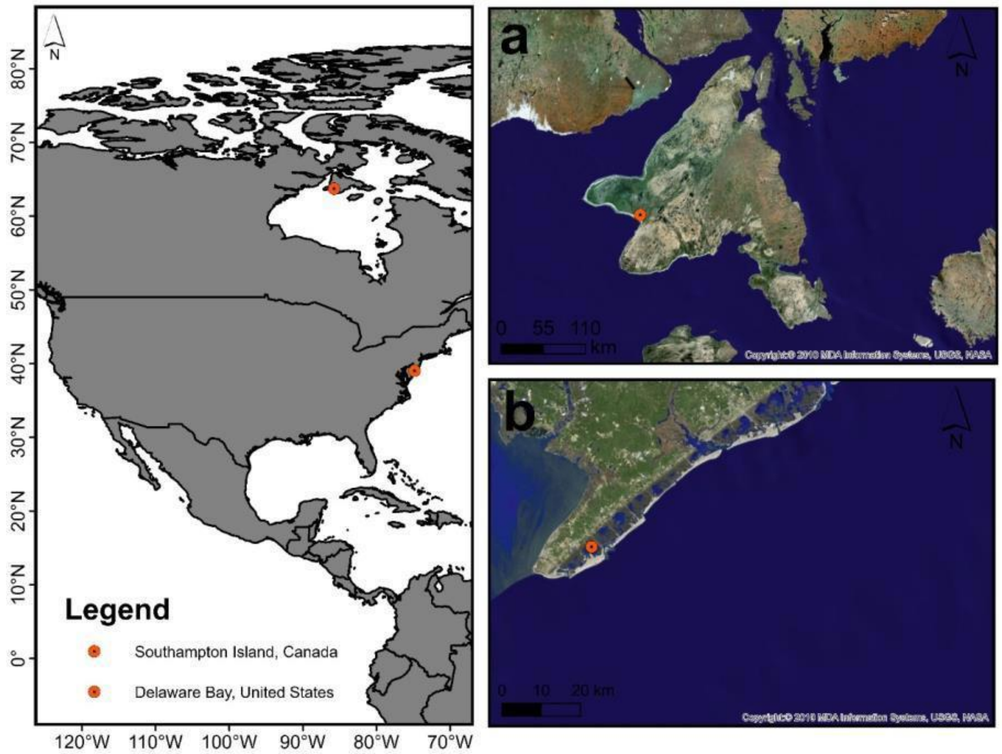



**Fig. 5** - Areas accessed by the E7X bird: a) Breeding area in Southampton Island – Cana b) Stopover in Delaware Bay, New Jersey - USA.

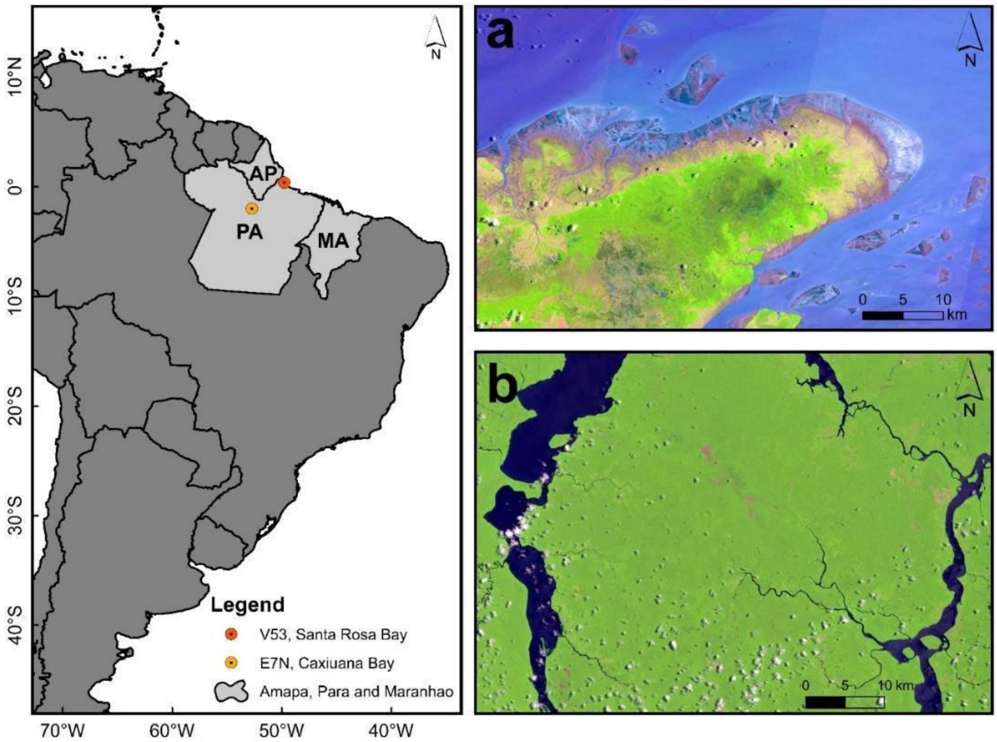



**Fig. 6** - Areas in the state of Pará without previous registration for Red Knots, accessed by birds V53 and E7N , respectively. a Santa Rosa Bay); b)Xingu River and Caxiuanã Bay . AP = Amapá; PA = Pará; MA = Maranhão.

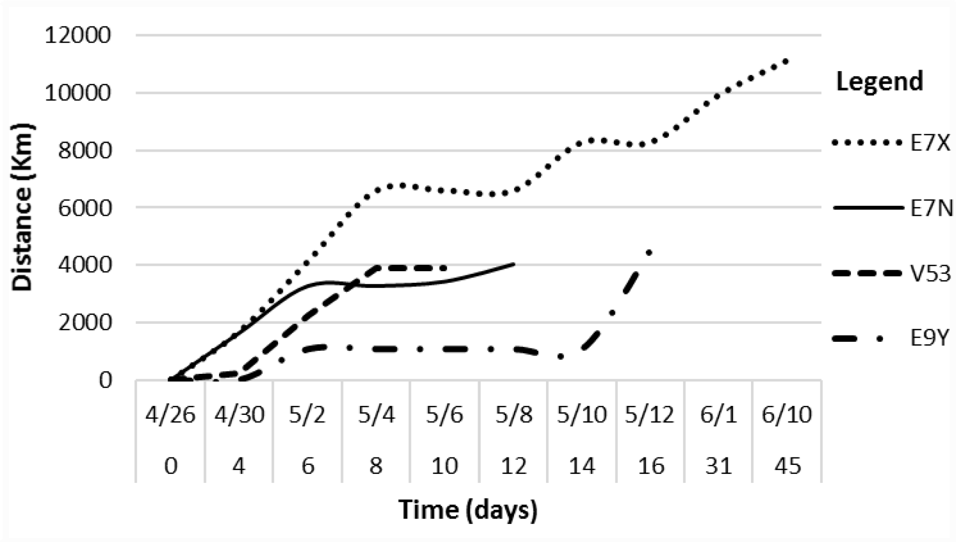



**Fig. 7**– Distances covered and days that each bird took to travel.

| **Birds** | **V53** | **E7X** | **E7N** |
|---|---|---|---|
| **Mass(g)** | 233 | 223 | 208 |
| **Wing(cm)** | 173 | 173 | 168 |
| **Days** | 4 | 10 | 6 |
| **Distance cover (Km)** | 3.647 | 6.599 | 3.280 |
| **Migration speed(Km/d)** | 1014 | 1229 | 817 |

**Table 3**: Relationship between physical condition and distances and time taken to travel.

## DISCUSSION

The results shown different migration strategies among *Calidris canutus rufa* individuals. Among the five birds analyzed, four migrated, each using different routes. Despite the varied paths, two major routes have already been used by migratory birds within Brazil: the central Brazil route (Pantanal, Amazon) and the Atlantic coast route (Baker *et al*. 2005; Niles *et al*. 2010; Oliveira *et al*. 2016; Brow *et al*. 2017). Differences in migration strategies within the same population are already reported (Alves et al., 2016). There are many hypotheses for this to happen. The individuals' body mass and unfavorable climatic changes, e.g. very strong winds or cyclones, which occur during migration can wield pressure on the direction of birds along their migratory journey (Bathley *et al*. 2012, Johson *et al*. 2011, Covino *et al*. 2015). However, since the Red Knot males assist the newborns at least two weeks after hatching and the juveniles follow the adults in the first migration, it can also be a type of behavioral inheritance (Leyrer *et al.* 2012). On the other hand, the routes variation between the knots can be explained in part by the native philopatry, quantity and quality of prey existing in the foraging sites and by the risk of predation in the stopovers (Bauer *et al*. 2010).

However, the reasons behind the choice of certain stopovers are still not well understood for Red Knots (Piersma *et al*. 2007, Niles *et al*. 2009, Andres *et al*. 2012). What is known is that these birds are very dependent and faithful to the places where they stop, where they replenish the energy costs of flights, are able to rest from their days and are conditioned to continue their migration to the breeding or wintering regions (Bathley *et al*. 2012, Newstead *et al*. 2013).

***Migration parameters and connectivity of environments***

Along the Atlantic coast there are many places that the *C. c. rufa* can make short stops to forage. Migratory bird species play a key role in the connectivity of these environments used as stopovers (Morrison *et al*. 2004, Lyons *et al*. 2016, Finch *et al*. 2017). Our study identified two main connections from Lagoa do Peixe, the Maranhão reentrances (Brazil) and the Delaware Bay (USA). Another environment has not yet been identified in the state of Pará in Santa Rosa Bay, mouth Amazonas River.

The E7X bird established a direct connection between Lagoa do Peixe and Delaware Bay on a direct flight, covering approximately 8,300 km in seven days. Although the first fix in Delaware was registered only on 10 May, we estimate that the bird arrived earlier. Based on the average flight speed (51 km/hr) calculated between its first (Mato Grosso, on 30 Apr) and second fix records (high seas, close to the Bahamas Islands, on 2 May), we estimate that E7X has left Lagoa do Peixe on 29 Apr and arrived in Delaware on 6 May. The average speed measured for this individual is within the average speed of 50 to 60 km/h already observed for long-distance migratory bird flights (Hassel *et al*. 2011, Newstead *et al*. 2013, Bishop *et al*. 2016). Our estimate is also consistent with previous studies, which found that an 8,000 km route, from southern Brazil to the North Carolina coast, was covered in 6 days (Niles *et al*. 2010). Similarly, assuming the estimated arrival date, E7X can be considered to have stayed in Delaware Bay for a period longer than six days, as recorded by GPS. However, the estimated number of days spent on the stopover is compatible with the number of days observed in previous studies (1 to 19 days) (Newstead *et al*. 2013, Mc Keller *et al*. 2015). The arrival date in Delaware (May 6th), also matches other observed ones, where Knots start arriving in early May and depart after 26 May (Niles *et al*. 2012, Burger *et al*. 2018). Interannual differences in arrival time in Delaware Bay are recurrent and may be related to variations in foraging conditions in previous stopovers or to unfavorable weather conditions during migration (Gonzales *et al*. 2006). After leaving Delaware Bay, E7X made another stop at James Bay (Canada), a stopover known to Red Knots and from which birds can fly directly to Hudson Bay (Newstead *et al*. 2013, Niles *et al*. 2012, Andres *et al*. 2012). The travel time between Delaware Bay and James Bay recorded by PinPoint was 16 days.However, this time is much longer than the bird would actually take to cover a distance of 1,658 km. The nine day time to cover the distance between James Bay and Southampton Island is also excessive. In general, Red Knots typically take 2 to 3 days to complete these routes (Newstead et al. 2013, Andres et al. 2012). It is possible that these excessively long times measured on these paths are related to the loss of position recording on the dates programmed in PinPoint's schedule, for position capture. That in the period between May 18th and May 31st, it was scheduled to send a position every five days, see table 01. Obviously, the loss of a position, due to some equipment failure, would represent the passage of 10 days. . The bird's last stop was in the south of Southampton Island and it is within the areas used for breeding (Newstead *et al*. 2013, Lathrop *et al*. 2018). Therefore, the bird E7X connected the Lagoa do Peixe - Delaware Bay - James Bay - Southampton Island, well-known environments for species, however the direct connectivity between the Lagoa do Peixe and Delaware Bay, makes these two stopovers essential for this subspecies, where conservation efforts should be focused.

The E7N bird, connected Lagoa do Peixe with the Maranhão recesses, also known as an important stopover for the *C. c. rufa* migrants from the south of Brazil and Tierra del Fuego and for a population that winters there (Baker *et al*. 2005, Niles *et al*. 2008, Andres *et al*. 2012). E7N used unpublished areas for the species, one between the Amazon River and the Xingu River, in the Green Forever Extractive Reserve and another in the Caxiuanã National Forest reserve, in the southern portion of Caxiuanã Bay, both in the state of Pará. The bird would have traveled around 3,280 km, from Lagoa do Peixe to stop in this area. It is possible to admit the use of this environment since two fixes are recorded on the site, indicating that she spent at least four days circulating in the area before heading to the Maranhão recesses, where she stopped for another 14 days. The long period of stay can also be linked to the increase in the rates of ingestion of individuals, which are regulated by the availability and quality of the prey that is found in the places where they stop and the absorption capacity of the food (Battley *et al*. 2001). The bird also performs reverse movements in the migration and moves through different environments as soon as it interrupts its flight, covering short distances between 156 km and 45 km and longer distances of 670 km, in the same region. This behavior, in general, is linked to the exploration of the environment in the increase of ingestion rates (Hernandez *et al*. 2010). It is important to highligth that the E7N bird explores environments of extensive wetlands areas, whose characteristics are odds with the natural habitat of the specie and far from the interdict areas of sand on marine beaches, where it forages (Cohen *et al*. 2009; Carmona *et al*. 2013). However, a study of isotopic value in feathers of Red Knot, captured in the Bahia Lomas, Chile, demonstrated a low isotopic value ($\delta^{13}C$) due to the influence of continental waters, suggesting that birds can also use rivers and ponds, far from their natural habitat (Atkinson *et al*. 2005). When the bird is weakened during migration and is unable to move forward it can stop in the most favorable habitat selected (Bishop *et al*. 2016). On the other hand, staying for a very long time in the area is unusual and represents that the individual is delaying migration and exposing himself to risks in the area and compromising its reproductive success (Alves *et al*. 2016, Bishop *et al*. 2016). Long-distance migrants are more vulnerable to anthropogenic actions that may reflect on the individuals's fitness, because if fewer individuals manage to reach their breeding areas, as a result of the disturbances our other situation found in their migratory routes, the lesser number of descendants they can leave for the future generations and this may have a negative impact on the fitness of the population (BUCHAN *et al*. 2020). Usually migratory birds from long distances spend little time in the areas where they stop, especially when they go to breeding areas (Gonzales *et al*. 2006, Piersma *et al*. 2007).

Two monitored birds V53 and E9Y moved along the coast of Brazil, at least to the Southeast Region of Brazil, including the limit of registration of Red Knot through photos posted on Wikiaves (Wikiaves 2020, Somenzari *et al*. 2018), except records made in the Bahia State coast, during migration to wintering areas, one made in February 2020 (Abreu 2020) and two others in September 2017 (Cerqueira 2017), one of the birds of this last record carried a light green flag, indicating that it was ringed in Delaware Bay. Although the E9Y bird has only two recorded positions, considering the migration speed (345 km/day) it shows that it is not a direct flight, leading to consider that it has accessed the Maranhão recesses before beginning the crossing of the Caribbean Sea. We believe it has adopted the same migration strategy as V53, advancing in short flights along the Atlantic coast. This shift in short flights along the coast is indicated by the records of the first two fixes of the V53, recorded over a distance of 214 km, with two days of difference between one and the other. Clearly it would not take all this time to cover a course of this length, leading to the supposition that it traveled in short flights along the beach, until it made a long flight of 2,028 km, passing through the state of Bahia and going another 1,628 km, until reaching the Amazon River in the North of the State of Amapá - Santa Rosa Bay, staying at least two days in this area. We have no report or record for the species in this remote area, showing that this individual selected another stopover further north of Brazil and may indicate a change in the migratory strategy of *C. c. rufa*, which deserves to be investigated. However, it is perfectly predictable that the bird will be able to use this location, as it is located between areas where the bird usually stops, in the Maranhão state and on the coast of French Guianas and Suriname (Baker *et al*. 2004, Navedo *et al*. 2019). The distances of displacement of birds E7N, E9Y and V53 varying between 1,600km to 3,400km correspond with others already registered for the same species, 2,000Km (Bishop *et al*. 2016); 2,300km (Carmona *et al*. 2013); 3,300km (Niles *et al*. 2010). Medium-sized migratory birds such as Knots can fly long distances, averaging 5,000 to 8,000 kilometers (Niles et al, 2010; Brow *et al*. 2017, Hill & Renfrew 2019) or make small distances (Newstead *et al*. 2013). According to Niles *et al*. (2010) the Red Knots have as a migration strategy to make long distance flights from the south of Brazil and another short flights with stops in some existing places on the migratory route, which is corroborated with our study, where one bird flies directly to the USA and three make shorter flights and stops along the route, demonstrating the importance of the connectivity of these habitats that support birds along their migratory routes.

***The importance of Lagoa do Peixe National Park to Red Knot***

The importance of the Lagoa do Peixe National Park for migratory birds has been highlighted since 1988 by several authors (Lara-Rezende & Leeuwenberg 1987, Harrington *et al*. 1988, Vooren & Chiaradia 1990, Morrison *et al*, 2004, Niles et al. 2010; Petry *et al*. 2012, Lyons *et al*. 2018). This place also serves as a temporary residence for birds that, for some reason, interrupted the migration, as confirmed by the bird E8A. Several hypotheses are involved in the interruption of migration, among them: age, juvenile individuals, who have not completed the second molt, do not migrate due to the absence of flight feathers, supported by some authors as an evolutionary adaptation that increases the chances of survival of these individuals, in the first years of life (Reneerkens *et al*. 2020); poor physical condition, individuals which do not reach an ideal weight to migrate, do not migrate; the risks found in the stopovers, individuals who suffer chemical contamination by pollutants and the action of parasites can also interrupt the migration (Martínez-Curci *et al*. 2020). The coast of the State of Rio Grande do Sul is an area of intense rice cultivation associated with the application of pesticides and water changes for irrigation of crops. This activity favors the transport of pollutants into the ocean through the numerous drainage channels existing on the coast, leading to contamination of coastal environments (Lima *et al*. 2005, Stenert *et* *al*. 2012). Several Knots individuals have been registered debilitated or dead in the coast of Rio Grande do Sul since 2000 (Buehler *et al*. 2010, Fedrizzi 2008). Therefore, it is possible that the bird E8A suffered some contamination and weakened quickly after being tagged and did not migrate, since it has an ideal weight to migrate. The migration interruption can be deduced since throughout the monitoring period from April 26th to July 23rd, it was circulating at the height of the Lagoa do Peixe mouth. Certainly, after this date the bird would hardly migrate, since at the end of July the reproduction period of the species is already ending (Niles *et al*. 2008, Lyons *et al*. 2018, Lathrop *et al*. 2018). However, it shows the importance of the area for the species due to the permanence in the area and the time of use, even outside the reproductive period.

***Individuals physical condition***

In the present study, we observed that the physical conditions of the birds may have played an important role in the direction of flights during the migratory route or made they had a better performance in migration. Two monitored females, E7X and E7N, considering the registered fixes, must have left on the same day from the place where the PinPoints were tagged, following the Central Brazil route and at a given moment they separate from each other. The bird with the highest E7X mass 233 g and the longest wing length 173 cm made a flight, without stops, until reaching the Delaware Bay, while the E7N bird, with the lowest mass 208 g and lesser wing length 168 cm, made two stops at different locations in Northeast Brazil and far from the marine coastal. These differences in body mass and wing performance may have influenced the migration strategy adopted between these two individuals, although it is admitted that the birds are within the ideal weight condition to migrate, which varies from 180 - 220 g (Baker *et al*. 2004, Atkison *et al*. 2007). The affinity between body mass with time and travel distances has also been observed by Morrison *et al*. 2007. We assume, because of this, that the bird E7X, with better physical condition, traveled a long distance of 8,300 km, in direct flight, while the bird E7N after traveling 3,281 km stopped the flight in order to restore its stamina. The condition of this bird may also have forced it to stop far from the usual stopover, more than 750 km, taken six days to move to the coastal area, in the Maranhão recesses, an important area for migratory birds, especially for the *C. c. rufa,* where a population that does not migrate to the south also winter there (Baker *et al*. 2005, Escudero *et al*. 2012). Red Knots have a physiological system well adapted for long distance flights. As soon as they start the migration, their digestive system and gizzards are reduced and it takes some time after they stop, in a certain place, to reactivate these functions (Piersma *et al*. 1996, Morrison *et al*. 2007, Duijns *et al*. 2017). For this reason, stopping many times along the route can represent a very high energy cost for individuals, in addition to exposing themselves to risks during the stop or delaying the arrival to the reproductive areas too much and putting reproduction at risk.

Getting good conditioning in Lagoa do Peixe and migrating straight to Delaware Bay can be more advantageous for individuals, since in Delaware birds feed almost exclusively on horseshoe crab eggs, which are easily absorbed and do not need so much gizzard and in a short time increase their intake rates (Baker *et. al*. 2004). On the other hand, if the bird stops in Maranhão, it will need time to restore the size of the internal organs to be able to digest harder preys, especially bivalves that are swallowed whole and need the gizzard to break and digest this type of food (Van Gils *et al*. 2003). Therefore, in addition to the risks that are exposed, you will also have to deal with the costs of physiological changes for migration. So many variables involved can result in the interruption or delay of migration, changing the life cycle, or being exposed to some event that represents carry-over effects that will bring consequences for the population in the increase of recruitment rates (Harrison *et al*. 2010). Although it is complicated to establish differences in the analysis of such a low number of individuals, it stills represents an alert of the importance of studies focused on the physical conditioning of birds in the two stopover that are connected.

***PinPoint GPS Argos 75 considerations***

The specie *C. canutus* is excellent for carrying out studies with GPS equipment, since they usually stop in open areas, facilitating the reception of satellite signals due to the absence of physical barriers on the ground cover (Burger *et al*. 2012).

In this study, the same schedule was performed for all equipment to record 25 positions fixes, for each bird, however, we had a low utilization of recorded positions fixes. The E9Y bird, for example, had only two fixes registered, representing 7.7% of the total positions expected for this individual. Scheduling the agenda for recording positions at intervals of two days, three days and five days, may have been a complicating factor for our study. This is because, when a position is not captured, the agenda closes and will only make a new attempt in the next programming, remembering that the attempt that the tag takes to receive and record the 50 mm GPS antenna signal is 70 seconds (Scarpignato *et al*. 2017, Argos 2018). In our case, the initial attempts were every two days and if a position was not recorded, it would take four days until the second attempt, which for a bird that is in its migratory period and moving at a speed greater than 50 km/h can be a long time and there is a risk of missing important events such as a sudden stop somewhere, not yet registered for the species. The recommendation in order to achieve a better coverage of the entire path traveled by the bird is to program the schedule for recording two fixes per day or at least one per day.

Another interesting issue is related to the use of glue to attached the PinPoints in birds, this technique has already been used and tested in some studies (Burger *et al*. 2012, Alves *et al*. 2016, Bishop *et al*. 2016, Oudman *et al*. 2018) and there are no reported problems for bird health, nor estimates of equipment permanence time. In our study we observed that the equipment was lost by two birds in mid-July and three birds in mid-May, therefore, less than three months after the attachement of the PinPoints. This may be linked to the quality of the adhesion of the glue used. It is hoped that with modern technologies it will be possible to think of a glue that may have greater fixing power and prolong the time that the equipment remains attached to birds***.***

However, in this study there was no harm to the birds with the use of this methodology for attached the equipment, since they were sighted one year after theend of the position recordings sent by the PinPoint. These records are on the 'bandedbird.org website', which is a platform for postings marked bird resighting.

**CONCLUSION**

The data obtained from the PinPoints allowed to trace the migratory route of four individuals of *C. canutus rufa* and to identify places where these birds stop during their journeys, as well as to infer flight speed data, distances covered, time spent traveling to the along the migratory routes and calculation of stopping time at the stopover used on the route.

At the end of the study, it was possible to conclude that the red knots that use Lagoa do Peixe as a stopover, after leaving this place, follow different migratory routes, some birds travel along the route from central Brazil, flying straight to the Delaware Bay, connected by these two stopover and other birds follow the migration along the Atlantic coast route. However, on both routes, some birds make stops in Maranhão, in northeastern Brazil, which connects Lagoa do Peixe to the Maranhão recesses. These results reveal differences within the same population in the migration strategies adopted by individuals.

The study also revealed a new stopover or stop not yet mentioned in the literature, which was accessed by one of the birds, increasing the expectation for behavior change during the movement to the breeding areas.

It also reveals that individuals even able to migrate, for some reason, do not migrate and remain in Lagoa do Peixe, highlighting the importance of this habitat for the species under study.

Although it was not possible, due to the low number of monitored birds and also the small number of recorded fixes, to test the differences found by statistical methods, the study takes an important look at these particularities in the migration strategies adopted, demonstrating the importance of keeping investigating the migration of *C. canutus rufa* from Lagoa do Peixe.

***Acknowledgment***

This project was partly financed by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ) (No. 425884 / 2016-3) and by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) through a PROSUC Master's scholarship to Antônio Coimbra de Brum (Finance code 001). The authors would like to thank the Unisinos Graduate Program in Biology, its coordinator Dr. Larissa Rosa de Oliveira and its professors; to colleagues at Unisinos' Laboratory of Ornithology and Marine Animals; To Dra. Carmem Fedrizzi for her help in the field, and ringbirds. To Leonardo Dorneles for his assistance with the tests; Douglas Ribeiro da Silva for his help in preparing the map of the study area and for his partnership in the fields, To Marlon Ferraz for his partnership in the fields. And special thanks to William Hahn for the translation of the manuscript.

**REFERENCES**

**Abreu, J.A.** 2017. WA2519503, *Calidris canutus* (Linnaeus, 1758). *Wiki Aves - A Enciclopédia das Aves do Brasil*. Accessed 26 Feb 2021 at: http://www.wikiaves.com/2519503. [In Portuguese]

**Aldabe, J., P. Rocca, P. Gonzalez, D. Caballero-Sadi & A. Baker.** 2015. Migration of endangered Red Knots *Calidris canutus rufa* in Uruguay: important sites, phenology, migratory connectivity and a mass mortality event. *Wader Study 122*: 221-235.

**Alves, J.A., M.P. Dias, V. Méndez, B. Katrínardóttir & T.G. Gunnarsson.** 2016. Very rapid long-distance sea crossing by a migratory bird. *Scientific reports* 6: 1-6.

**Andrade, R., L.F.Martini & S. P. Dieguez.** 2003. *Parques Nacionais: Brasil*. Empresa das Artes. São Paulo. [in portuguese]

**Andres, B.A., P.A. Smith, R.G. Morrison, C.L. Gratto-Trevor, S.C. Brown & C.A. Friis.** 2012. Population estimates of North American shorebirds. *Wader Study Group Bulletin* 119: 178-194.

**Antas, T.Z.P. & I.L.S. Nascimento**. 1996. Analysis of Red Knot Calidris *canutus rufa* banding data in Brazil. Int. *Wader Studies* 8: 63-70.

**Argos, C.L.S.** 2018. *Argos user's manual*. Accessed 26 Feb 2021 at: https://www.argos-system.org.

**Atkinson, P.W., A.J. Baker, R.M. Bevan, N.A. Clark, K.B. Cole, P.M. Gonzalez & R.A. Robinson**. 2005. Unravelling the migration and moult strategies of a long-distance migrant using stable isotopes: Red Knot *Calidris canutus* movements in the Americas. *Ibis*. 147: 738-749.

**Atkinson, P.W., A.J. Baker, K.A. Bennet, N.A. Clark, J.A. Clark, K.B. Cole, A. Dekinga, A. Dey, S. Gillings, P.M. Gonzalez, K. Kalasz, C. Minton, J. Newton, T. Piersma, R.A. Robinson & H.P. Sitters.** 2007. Rates of mass gain and energy deposition in red knot on their final spring staging site is both time- and condition-dependent. *J. Appl. Ecol*. 44: 885-895.

**Baker, A.J., P. M. González, I.L. Serrano, W.R. Júnior, M.A. Efe, S. Rice,... & M.E. Echave**. 2005. Assessment of the wintering area of red knots in Maranhão, northern Brazil, in February 2005. *Bulletin 107 August*: 3.

**Baker, A.J., P.M. Gonzalez, T. Piersma, L.J. Niles, I. de Lima S.do Nascimento, P.W. Atkinson, P. Collins, N.A. Clark, C.D.T. Minton, M.K. Peck & G. Aarts**. 2004. Rapid population decline in red knots: fitness consequences of decreased refuelling rates and late arrival in Delaware Bay. *Proceedings of the Royal Society of London* B 271: 875-882.

**Battley, P.F., M.W. Dietz, T. Piersma, A. Dekinga, S. Tang & K. Hulsman**. 2001. Is long-distance bird flight equivalent to a high-energy fast? Body composition changes in freely migrating and captive fasting great knots. *Physiological and Biochemical Zoology* 74: 435-449.

**Bauer, S., B.J. Ens & M. Klaassen.** 2010. Many routes lead to Rome: potential causes for the multi-route migration system of Red Knots, *Calidris canutus islandica*. *Ecology* 91: 1822-1831.

**Bencke, G.A., G.N. Maurício, P.F. Develey & J.M. Goerck**. 2006. *Áreas Importantes para a Conservação das Aves no Brasil*. Parte I–Estados do Domínio da Mata Atlântica. SAVE Brasil, São Paulo. [In Portuguese]

**Bijleveld, A.I., J.A.Van Gils, J. Jouta & T. Piersma.** 2015. Benefits of foraging in small groups: an experimental study on public information use in red knots *Calidris canutus*. *Behavioural Processes***.** 117: 74-81.

**Birdlife International**. *Calidris canutus*. *The IUCN Red List of Threatened Species*. Version 2014.3. 2018. Accessed 05 May 2019 at: www.iucnredlist.org.

**Bishop, M.A., J.B. Buchanan., B.J. Mccaffery & J.A. Johnson**. 2016. Spring stopover sites used by the red nnot *Calidris canutus roselaari* in Alaska, USA: Connectivity between the Copper River Delta and the Yukon–Kuskokwim River Delta. *Wader Study* 123: 143-152.

**Brasil.** 1986. *Decreto nº 93.546, de 6 de novembro de 1986*. Planalto, Brasília. Accessed 30 Nov 2019 at: http://www.planalto.gov.br/ccivil_03/decreto/1980-1989/1985-1987/D93546.htm. [In Portuguese]

**Buchan, C., J.J. Gilroy, I. Catry & A.M. Franco**. 2020. Fitness consequences of different migratory strategies in partially migratory populations: A multi-taxa meta-analysis. *Journal of Animal Ecology* 89: 678-690.

**Buehler, D.M., L. Bugoni, G.M., Dorrestein, P.M. González, J., Pereira Jr, L., Proença,... & T. Piersma.** 2010. Local mortality events in migrating sandpipers (*Calidris*) at a staging site in southern Brazil. *Wader Study Group Bulletin* 117: 150-156.

**Burger, J., L.J. Niles, R.R. Porter, A.D. Dey, S. Koch & Gordon, C.** 2012. Migration and over-wintering of red knots (*Calidris canutus rufa*) along the Atlantic coast of the United States**.** *The Condor* 114: 302-313.

**Burge, C.A., C.M. Eakin, C.S. Friedman, B. Froelich, P.K. Hershberger, E.E. Hofmann & S.E. Ford.** 2014. Climate change influences on marine infectious diseases: implications for management and society. Annual review of marine science, 6: 249-277.

**Burger, J., L. Niles, C. Jeitner & M. Gochfeld.** 2018. Habitat risk: Use of intertidal flats by foraging red knots (*Calidris canutus rufa*), ruddy turnstones,(*Arenaria interpres*), semipalmated sandpipers (*Calidris pusilla*), and sanderling (*Calidris alba*) on Delaware Bay beaches. *Environmental research* 165: 237-246.

**Carmona, R., N. Arce, V. Ayala-Perez, A. Hernández-Alvarez, J.B. Buchanan, L.J. Salzer,... & J.E. Lyons**. 2013. Red Knot *Calidris canutus roselaari* migration connectivity, abundance and non-breeding distribution along the Pacific coast of the Americas. *Wader Study Group Bulletin* 120: 168-180.

**Chan, Y.C., M. Brugge, T.L. Tibbitts, A. Dekinga, R. Porter, R.H. Klaassen & T. Piersma.** 2016. Testing an attachment method for solar-powered tracking devices on a long-distance migrating shorebird. *Journal of Ornithology* 157: 277-287.

**Cerqueira, R. T. (2017).** WA2701890, Calidris canutus (Linnaeus, 1758). *Wiki Aves - A Enciclopédia das Aves do Brasil*. Accessed 26 Feb 2021 at: http://www.wikiaves.com/2701890.

**Cohen, J.B., S.M. Karpanty, J.D. Fraser, B.D. Watts & B.R. Truitt.** 2009. Residence probability and population size of Red Knots during spring stopover in the mid-Atlantic region of the United States. *The Journal of Wildlife Management* 73: 939-945.

**Cooper, N.W., M.T. Hallworth & P.P. Marra**. 2017. Light-level geolocation reveals wintering distribution, migration routes, and primary stopover locations of an endangered long-distance migratory songbird. *Journal of Avian Biology* 48: 209-219.

**Covino, K.M., R.L. Holberton & S.R. Morris**. 2015. Factors influencing migratory decisions made by songbirds on spring stopover. *Journal of Avian Biology* 46: 73-80.

**D'amico, V.L., P.M. González, R.G. Morrison & A.J. Baker**. 2014. Reverse movements of Red Knots *Calidris canutus* during northward migration in Argentina. *Ardeola* 61: 63-76.

**Icmbio & Cemave**. 2020. *Manual de Anilhamento de Aves Silvestres*. Organizadores: Antônio Emanuel Barreto Alves de Sousa e Patrícia Pereira Serafini. 3ª ed. rev. e ampl. ICMBio/Cemave. Brasília. Accessed 26 Fev 2021 at: https://www.gov.br/icmbio/pt-br. [In Portuguese]

**Duijns, S., L.J. Niles, A. Dey, Y. Aubry, C. Friis, S. Koch,... & P.A. Smith**. 2017 Body condition explains migratory performance of a long-distance migrant. *Proceedings of the Royal Society B: Biological Sciences* 284: 20171374.

**Escudero, G., J.G. Navedo, T. Piersma, P. De Goeij & P. Edelaar**. 2012. Foraging conditions ‘at the end of the world’in the context of long-distance migration and population declines in red knots. *Austral Ecology* 37: 355-364.

**Fedrizzi, C.E. 2008**. *Abundância, distribuição e ecologia de forrageio de ave costeira (Charadriiformes: Charadrii and Scolopaci) na Costa do Rio Grande do Sul, Brasil*. Tese de doutorado, defendida na Universidade Federal de Rio Grande, Rio Grande.

**Finch, T., S.J. Butler, A.M. Franco & W. Cresswell**. 2017. Low migratory connectivity is common in long-distance migrant birds. **Journal of Animal Ecology** 86: 662-673.

**Frazier, S.** 1996. Visión general de los sitios Ramsar en el mundo. *Wetlands International* 39: 58. [In Spanish]

**Gaunt, G.S., L.W. Oring, K.P. Able, D.W. Anderson, L.F. Baptista, J.C. Barlow & J.C. Wingfield**. 1999. *Recomendações para o uso de aves silvestres em pesquisa.* Museu de Ciências e Tecnologia, Pucrs. Porto Alegre. [In Portuguese]

**González, P. M., A.J. Baker & M.E. Echave.** 2006. Fundación Inalafquen. Annual survival of Red Knots (*Calidris canutus rufa*) using the San Antonio Oeste stopover site is reduced by domino effects involving late arrival and food depletion in Delaware Bay. *El hornero*.

**Harrington, B. A., J.M. Hagan & L.E. Leddy.** 1988. Site fidelity and survival differences between two groups of New World Red Knots (*Calidris canutus*). *The Auk* 105: 439-445.

**Harrington, B.A., P.T.Z. Antas & F. Silva**. 1986. Northward shorebird migration on the Atlantic coast of southern Brazil. *Vida Silvestre Neotropical* 1: 45-54.

**Harrington, B.A., S. Loch, L. Niles & K. Kalasz.** 2010. Red Knots with different wintering destinations: differential use of an autumn stopover area. *Waterbirds* 33: 357-363.

**Harrison, X.A., J.D. Blount, R. Inger, D.R. Norris & S. Bearhop**. 2011. Carry-over effects as drivers of fitness differences in animals. *Journal of Animal Ecology* 80: 4-18.

**Hassell, C, I. Southey, A. Boyle & H.Y. Yang.** 2011. Red knot *Calidris canutus*: subspecies and migration in the East Asian-Australasian flyway–where do all the red knot go. *Birding Asia* 16: 89-93.

**Hernández, M.L.Á., L.R. Musmeci & L.O. Bala**. 2010. Optimización del tiempo de alimentación por parte del Playero Rojizo (*Calidris canutus rufa*) en Península Valdés, Patagonia Argentina. *Ornitologia Neotropical* 21: 445-451. [In Spanish]

**Hill, J.M. & R.B. Renfrew.** 2019. Migratory patterns and connectivity of two North American grassland bird species. *Ecology and Evolution* 9: 680-692.

**Icmbio. 2016**. *Relatório anual de rotas e áreas de concentração de aves migratórias no Brasil.* Cabedelo, PB: CEMAVE/ ICMBio, Brasília. [In Portuguese]

**Johnson, O.W., L. Fielding, J.W. Fox, R.S. Gold, R.H. Goodwill & P.M. Johnson**. 2011. Tracking the migrations of Pacific Golden-Plovers (*Pluvialis fulva*) between Hawaii and Alaska: New insight on flight performance, breeding ground destinations, and nesting from birds carrying light level geolocators. *Wader Study Group Bull* 118: 26-31.

**Lara-Resende, S. & F. Leeuwenberg**. 1987. Ecological studies of Lagoa do Peixe. Final Report to WWF-US, *Washington* 1: 52.

**Lathrop, R.G., L. NILES, P. SMITH, M. PECK, A. DEY, R. SACATELLI & J. BOGNAR**. 2018. Mapping and modeling the breeding habitat of the Western Atlantic Red Knot (*Calidris canutus rufa*) at local and regional scales. *The Condor: Ornithological Applications* 120: 650-665.

**Leyrer, J., T. Lok, M. Brugge, A. Dekinga, B. Spaans, J.A. Van Gils, ... & T. Piersma**. 2012. Small-scale demographic structure suggests preemptive behavior in a flocking shorebird. *Behavioral Ecology* 23: 1226-1233.

**Lima, A.P.S., J.E.S. Sarkis, H.M. Shihomatsu & R.C.S. Müller.** 2005. Mercury and selenium concentrations in fish samples from Cachoeira do Piria Municipality, Para State, Brazil. *Environmental Research* 97: 236-244.

**Lyons, J.E., W.L. Kendall, J.A. Royle, S.J. Converse, B.A. Andres & J.B. Buchanan**. 2016. Population size and stopover duration estimation using mark–resight data and Bayesian analysis of a superpopulation model. *Biometrics* 72: 262-271.

**Lyons, J.E., B. Winn, T. Keyes & K.S. Kalasz.** 2018. Post-breeding migration and connectivity of red knots in the Western Atlantic. *The Journal of Wildlife Management* 82: 383-396.

**Martínez-Curci, N.S., J.P. Isacch, V.L. D'amico, P. Rojas & G.J. Castresana.** 2020. To migrate or not: drivers of over-summering in a long-distance migratory shorebird. *Journal of Avian Biology* 51: 10.

**Mckellar, A.E., R.K Ross, R.G.Morrison, L.J. Niles, R.R. Porter, J. Burger, ... & P.A. Smith.** 2015. Shorebird use of western Hudson Bay near the Nelson River during migration, with a focus on the Red Knot. *Wader Study* 122: 201-211.

**Morrison, R.I.G. & R.K. Ross.** 1989. *Atlas of Neartic Shorebirds on the coast of South America*, Canadian Wildlife Service, Ottawa.

**Morrison, R.I., R.R. Kenyon & L.J. Niles**. 2004. Declines in wintering populations of Red Knots in southern South America. *The Condor* 106: 60-70,

**Morrison, R.I., N.C. Davidson & J.R. Wilson.** 2007. Survival of the fattest: body stores on migration and survival in red knots *Calidris canutus* islandica. *Journal of Avian Biology*, v. 38, n. 4, p. 479-487,

**Navedo, Juan G., Gutiérrez, Jorge S.** Migratory connectivity and local site fidelity in red knots on the southern Pacific coast of South America. **Aquatic Conservation:** *Marine and Freshwater Ecosystems*, v. 29, n. 4, p. 670-675, 2019.

**Newstead, D.J., L.J. Niles, R.R. Porter, A.D. Dey, J. Burger, & O.N. Fitzsimmons**. 2013. Geolocation reveals mid-continent migratory routes and Texas wintering areas of Red Knots *Calidris canutus rufa*. *Wader Study Group Bulletin* 120: 53-59.

**Niles, L.J., H.P. Sitters, A.D. Dey, P.W. Atkinson, A.J. Baker, K.A. Bennett, ... & I.L. Serrano.** 2008. Status of the Red Knot (*Calidris canutus rufa*) in the Western Hemisphere. In: *Status of the Red Knot (Calidris Canutus Rufa) in the Western Hemisphere 2008*: 1-185.

**Niles, L.J., J. Bart, H.P. Sitters, A.D. Dey, K.E. Clark, P.W. Atkinson, ... & J. Clark**. 2009. Effects of horseshoe crab harvest in Delaware Bay on red knots: are harvest restrictions working? *BioScience* 59: 153-164.

**Niles, L.J., J. Burger, R.R. Porter, A.D. Dey, C.D. Minton, P.M. González & C. Gordon.** 2010. First results using light level geolocators to track Red Knots in the Western Hemisphere show rapid and long intercontinental flights and new details of migration pathways. *Wader Study Group Bull* 117: 123-130.

**Niles, L.J., J. Burger, R.R. Porter, A.D. Dey, S. Koch, B. Harrington, & M. Boarman**. 2012. Migration pathways, migration speeds and non-breeding areas used by northern hemisphere wintering Red Knots *Calidris canutus* of the subspecies rufa. *Wader Study Group Bull* 119: 195-203.

**Oliveira, A.D., A.E.A. Barbosa, A.E.B.A. Sousa, C. Lugarini, D.M. Lima, J.L.X. Nascimento, ... & P.P. Amaral.** 2016. *Relatório anual de rotas e áreas de concentração de aves migratórias no Brasil*. **CEMAVE/ICMBio, Cabedelo**. Brasília. [In Portuguese]

**Oudman, T., T. Piersma, M.V.A. Salem, M.E. Feis, A. Dekinga, S. Holthuijsen, ... & A. I. Bijleveld.** 2018. Resource landscapes explain contrasting patterns of aggregation and site fidelity by red knots at two wintering sites. *Movement ecology* 6: 1-12.

**Petry, M.V., J.D.F.M. Scherer & A.L. Scherer**. 2012. Ocorrência, alimentação e impactos antrópicos de aves marinhas nas praias do litoral do Rio Grande do Sul, sul do Brasil. *Revista Brasileira de Ornitologia* 20: 65-70.

**Piersma, T., L. Bruinzeel, R. Drent, M. Kersten, J. Van Der Meer, & P. Wiersma.** 1996. Variability in basal metabolic rate of a long-distance migrant shorebird (red knot, *Calidris canutus*) reflects shifts in organ sizes. *Physiological Zoology* 69: 191-217.

**Piersma, T., D.I. Rogers, P.M. González, L. Zwarts, L.J. Niles, I.L.S. Nascimento, ... & A.J. Baker.** 2005. Fuel storage rates before northward flights in Red Knots worldwide. *Birds of Two Worlds: the ecology and evolution of migration* 262-273.

**Piersma, T**. 2007. Using the power of comparison to explain habitat use and migration strategies of shorebirds worldwide. *Journal of Ornithology* 148: 45.

**Reneerkens, J., T.S. Versluijs, T. Piersma, J.A. Alves, M. Boorman, C. Corse, ... & T. Lok.** 2020. Low fitness at low latitudes: Wintering in the tropics increases migratory delays and mortality rates in an Arctic breeding shorebird. *Journal of Animal Ecology* 89: 691-703.

**Scarpignato, A.L., A.L. Harrison, D.J. Newstead, L.J. Niles, R.R. Porter, M. Van Den Tillaart & P.P. Marra.** 2016. Field-testing a new miniaturized GPS-Argos satellite transmitter (3.5 g) on migratory shorebirds. *Wader Study* 123: XX-XX.

**Scherer, A.L. & M.V. Petry.** 2012. Seasonal Variation In Shorebird Abundance In The State of Rio Grande Do Sul, Southern Brazil. *The Wilson Journal of Ornithology* 124: 40-50.

**Somenzari, M., P.P.D. Amaral, V.R. Cueto, A.D.C. Guaraldo, A.E. Jahn, D.M. Lima, ... & J.L.D. Nascimento.** 2018. An overview of migratory birds in Brazil. *Papéis Avulsos de Zoologia* 58.

**Stenert, C., R.C. Bacca, A.B. Moraes, A.C. Ávila & L. Maltchik.** 2012. Negative effects of exotic pine invasion on macroinvertebrate communities in southern Brazil coastal ponds. *Marine and Freshwater Research* 63: 283-292.

**Van Gils, J.A., T. Piersma, A. Dekinga, & M.W. Dietz.** 2003. Cost-benefit analysis of mollusc-eating in a shorebird II. Optimizing gizzard size in the face of seasonal demands. *Journal of experimental biology* 206: 3369-3380.

**Vooren, C.M. & A. Chiaradia.** 1990. Seasonal abundance and behaviour of coastal birds on Cassino Beach, Brazil. *Ornitologia neotropical* 1: 9-24.

**Wikiaves**, 2020. *A Enciclopédia das Aves do Brasil.* Asseced 27 Jun 2020 at: www.wikiaves.com. [In Portuguese]

# CAPÍTULO II

## HABITAT USE OF RED KNOTS *Calidris canutus rufa* IN THE MIDDLE COAST OF RIO GRANDE DO SUL, EXTREME SOUTHERN BRAZIL

(O manuscrito a seguir foi elaborado nas normas de estilo da revista **Wader Study Bolettin** exceto no espaçamento das linhas, justificação do texto e locais de posicionamento das figuras, com o fim de auxiliar na revisão do texto e resultados pela banca examinadora.)

# HABITAT USE OF RED KNOTS *Calidris canutus rufa* IN THE MIDDLE COAST OF RIO GRANDE DO SUL, EXTREME SOUTHERN BRAZIL


## ABSTRACT

The middle coast of Rio Grande do Sul, extreme south of Brazil, is a stopover of the subspecies *Calidris canutus rufa* on both its north and south bound migration along the Western Atlantic flyway. This stopover site is characterized by littoral ponds, wetlands, extensive dune areas, with the presence of drainage channels and wide sandy dissipative beaches, constituting an ideal environment for shorebirds. Flock counts of Red Knots were conducted between 2019 and 2020 in order to analyze habitat use by the subspecies during its migratory stopover and determine which environmental variables best explain the pattern of presence of the birds in certain locations. We found significant differences between the size and distance of Red Knot flocks during different migration directions. In northward migration, the flocks are larger and maintain greater distance from each other, and in southward migration, the opposite occurs. We also found differences in the pattern of occurrence and distribution of flocks along the latitudinal gradient of the beach, related to the different variables analyzed. During the northward migration, the formation of two clusters with distinct environmental preferences was detected. The southernmost cluster flocks appeared to be influenced by the size of the pond area and wetland and the northernmost cluster flocks at a distance from ponds and wetlands. During the southward migration, four clusters were identified, two of which appeared to be influenced by distance from ponds and distance from drainages and urban areas. The distribution of flocks is structured in areas that have greater similarity of variables. The variables size and distance of ponds, and wetlands were the variables that best responded to the ordering pattern of the presence of flocks in the habitat, as well as the presence of drainages, size and distance from urban areas, had a complementary role in the structuring of flocks of Red Knot. Flock size and distancing is a behavior that reduces predation risks and reflects the degree of disturbance that the species may be experiencing in its stopovers. Habitat use studies are crucial to identifying environments that are critical to the species' viability and that must be protected.


**Key-words:** Environment. Wetland. Ponds. Migration. Shorebirds.

## RESUMO

O Litoral Médio do Rio Grande do Sul, extremo sul do Brasil, é um *stopover* da subespécie *calidris canutus rufa* durante sua migração. Este *stopover* é caracterizado por possuir lagoas litorâneas, áreas úmidas, extensas áreas de dunas, com presença de canais de drenagem e amplas praias dissipativas arenosas, constituindo ambiente ideal para aves limícolas. Contagens de bandos de Red Knots foram realizadas entre 2019 e 2020 para analisar o uso do habitat pela subespécie durante sua parada migratória e determinar quais variáveis ambientais melhor explicariam o padrão de presença das aves em determinados locais. Encontramos diferenças significativas entre o tamanho e a distância dos bandos de Red Knot durante diferentes direções de migração. Na migração para o norte, os bandos são maiores e mantêm maior distância uns dos outros, e na migração para o sul ocorre o contrário. Também encontramos diferenças no padrão de ocorrência e distribuição dos bandos ao longo do gradiente latitudinal da praia, em relação às diferentes variáveis analisadas. Durante a migração para o norte, foi observado a formação de dois agrupamentos com preferências ambientais distintas. Os bandos agrupados mais ao sul pareceram ser influenciados pelo tamanho da área das lagoas e áreas úmidas e os bandos agrupados mais ao norte pela distância de lagoas e áreas úmidas. Durante a migração para o sul, quatro agrupamentos foram identificados, dois dos quais pareceram ser influenciados pela distância das lagoas e distância das drenagens e áreas urbanas. A distribuição dos bandos está estruturada em áreas que apresentam maior similaridade de variáveis. As variáveis tamanho e distância de lagoas e áreas úmidas foram as variáveis que melhor responderam ao padrão de ordenação da presença de bandos no habitat, bem como a presença de drenagens, tamanho e distância das áreas urbanas, tiveram um papel complementar na estruturação dos bandos de Red Knot. O tamanho e o distanciamento dos bandos é um comportamento estratégico que reduz os riscos de predação e reflete o grau de perturbação que a espécie pode estar experimentando em seus locais de parada. Estudos de uso de habitat são cruciais para identificar ambientes críticos para a viabilidade da espécie e que devem ser protegidos.

**Palavra chave:** Ambiente. Área úmida. Lagoas. Migração. Aves limícolas.

**INTRODUCTION**

The Red Knot *Calidris canutus* is a migratory bird in the Scolopacidae family. The annual migration of the Western Atlantic flyway subpopulation, *C. c. rufa*, begins in the Canadian Arctic, where it breeds between June and July, and ends in Tierra del Fuego in southern South America, passing through Brazil (Niles *et al*. 2008, Cohen *et al*. 2009, Gilroy *et al*. 2016, Lathrop *et al*. 2018).

The populations of *C. c. rufa* present two migration strategies with both short and the long distance migrants. The shorter distance migrants migrate to the Gulf of Mexico the northern coast of South America (Maranhão, Brazilian´s state), and in the longer distance group, the birds fly all the way to Tierra del Fuego, the extreme south of Argentina and Chile (Baker *et al.* 2004, Newstead *et al*. 2013, Lambe *et al*. 2018). Due to the energy expenditure of their long flights, Red Knots depend on the existence of environments with an abundance of food and resting areas along their migratory routes (Harrington *et al*. 2007, 2010, Burger *et al*. 2015).

During long-distance migration, Red Knots stop along the middle coast of Rio Grande do Sul and the Lagoa do Peixe National Park, Brazil, as an area for foraging, resting, and post-reproductive feather changes (Scherer & Petry 2012, NILES *et al*. 2010). The Lagoa do Peixe and surroundings, therefore, are classified as an important stopover, or stopping area, for the subspecies (Harrington *et al*. 1988). The passage of the Red Knot occurs during the migration to the north in late March and early April and in the migration towards the south, in the months of September and October (Harrington *et al*. 1988, Scherer & Petry 2012, Niles *et al* 2008).

Several researchers have suggested that Red Knots employ different stopover strategies on their northward migration (i.e., towards their breeding areas) vs. their southbound migration to overwintering areas (Andres *et al* 2012, Burger *et al.* 2012, Lyons *et al*, 2018). One of these strategies is in the way the species uses the habitat. Usually when it is migrating to the breeding areas the birds spend less time on their stopovers, but maintain a high fidelity to these foraging and resting places (Gonzáles *et al*, 2006, Niles *et al*. 2010, Andres *et al*, 2012). When the bird migrates to the wintering areas it disperses more and explores more environments (Piersma 2007, Harrington *et al* 2010, Bishop *et al*, 2016).

Outside their breeding grounds, Red Knots use intertidal zones of sandy and muddy beaches to eat and adapt its diet according to the availability of resources in the stopovers (Niles *et al*. 2008, Burger *et al*. 2018). In the wintering and stopover areas, small mollusks, gastropods, and polychaetes, which occur in the sweep of the beach and in marsh formation sites, constitute the majority of the species' diet (Harrington 1988, Gonzáles *et al*. 1996, Niles *et al.* 2008). These are removed from the soft sand using its beak adapted for this purpose (Piersma 2007).

In addition to exploring the intertidal areas of the beaches, which are their primary environment, Red Knots also use sandbanks, freshwater, and brackish ponds, river slopes, and freshwater drainage channels that flow into the sea (Cohen *et al*. 2010, Catry *et al*. 2015, McKellar *et al*. 2015, Burger *et al*. 2018). Although it is a coastal species, they can use wetlands and freshwater locations, close to coastal areas, for maintenance of feathers and for supplementary feeding, especially when the preferred areas are suffering some type of degradation (Burger *et al*. 2012, Aldabe *et al*. 2015).

Intertidal zones have high productivity and, therefore, attract several bird species, reflecting an increase in direct and indirect interspecific competition and the availability of resources (Aldabe *et al,* 2015, Musmeci, *et al.* 2012). In addition to the competition, other factors may interfere with the availability of resources. On a larger scale of time, climate change, and on a smaller scale, the humam alteration/degradation of environments (Fraser *et al*. 2013). One of the challenges for the species is to find available resources to replace the energy costs of the previous flight and accumulate enough energy to start a new journey during the short time window that remains on the stopover (Gonzáles *et al*, 2006, Niles *et al*. 2008, Andres *et al*. 2012).  This need subjects the species to the selection of locations, along its migratory route, where food is available and abundant. (Buehler *et al*. 2010).

Red Knots are gregarious birds that are grouped in small or large flocks to forage. Flocks are cohesive and made up of dominant and non-dominant individuals and these flocks maintain distances between individual flocks (Bijilevel *et al*. 2012). This behavior assists in the sharing of community information in locating prey, reducing the risks of predation, and helps in moderating competition with other species (Bijilevel *et al*. 2015).

The characteristics of the environment are essential for the selection and use of the habitat since they will influence the distribution of prey and demonstrate the degree of humam disturbance and environmental changes. Many studies have performed remote monitoring by tracking (Cohen *et al*. 2010, Newstead *et al*. 2013), analysis of prey distribution (Musmeci *et al*. 2012), and analysis of stable carbon and nitrogen isotopes in feathers in the investigation of the habitat use of *Calidris canutus* (Atkinson *et al*. 2005, Catry *et al*. 2015). However, few have dedicated themselves to describing the *in situ* characteristics of the environment that determine the selection and use of habitat in stopovers (Roesler & Imberti 2005, Bishop *et al*. 2016). Here we examine whether during the northward and southward migration the long-distance migrant population of the subspecies *C. c. rufa* exhibit a pattern in the habitat use along the coastal strip of the middle coast of Rio Grande do Sul and whether the presence of birds in certain locations can be explained by environmental variables. So, considering the way the Red knot individuals group together to increase the chances of finding prey and reduce the risk of predation, as well as that during southward migration birds are more dispersed than when they are migrating northward, we expect that the size of the flocks and spacing between them occur differently between the two migration directions. We also expect the occurrence and distribution of C. c. rufa, along the latitudinal gradient of the interdital beach strip, are influenced by environmental variables prevalent in certain areas along the coast and by variables related to the size of the flocks and the distance between them.

## METHODOLOGY

### *Study Area*

On the middle coast of Rio Grande do Sul, a stretch of 150 km, from the municipality of Balneário Pinhal to Lagoa do Peixe´s inlet in Mostardas (30º14´34´´S, 50º13´.36´´W: 31º21´34´´S,51º02´26´´W) (Fig. 1). This portion of the coast is characterized by fixed and migratory frontal dunes and a coastal area with the presence of a complex of pons and wetlands (Fig. 2a,b) (Toldo *et al*. 2006). The Middle Coast has a long stretch of beaches, with low waves and a large wave breaking region, allowing the wave energy to dissipate. The width of the beach varies between 50 and 120 meters and has low dunes and fine sand, in almost its entire length. (Fig. 2c) (Mota *et al*. 2015). The tide varies on average 0.40m only increasing due to strong winds, especially in the winter period with cold fronts that advance from the south, which also alter the wind regime that is predominantly northwest (Toldo *et al*. 2006, Mota *et al*. 2015). The middle coast has a large number of drainage streams that drain directly from the adjacent uplands and bisect the beach strand carrying sediments and nutrients to coastal ocean waters (Mota *et al*, 2015). Some of these streams are permanent while others only flow during times of high precipitation, when the accumulated excess water breaks the dune barrier and forms new runoff channels. (Fedrizzi, 2008) These drainage channels form braided channels and mini-deltas, increasing the zone of the beach. These locations serve as shelter, roosting, foraging, i.e. act as secondary habitat (Fig. 2d,e) and can attract many birds (Scherer & Petry, 2012). Among them are representatives of the families Scolopacidae (sandpipers), Charadriidae (plovers), and Sternidae (terns). Migrant birds from temperate or subarctic regions of North America and from southern parts of South America (Belton, 2000, Bencke *et al*. 2006).

The Lagoa do Peixe National Park, with approximately 34,400 ha, covers the municipalities of Tavares and Mostardas, between coordinates 31º04´S, 51º09´W : 31º29´S, 50º46´W (Fig. 1) on the South Coast of Brazil. The Park includes a complex of coastal lagoons, tidal marsh, dunes and barrier beach (Oliveira *et al*. 2016). It is a fully protected Conservation Unit created in 1986 (Brasil, 1986). It represents an important hotspot, especially for migratory birds, internationally recognized as a Biosphere Reserve Site by the Ramsar Convention, to which Brazil is a signatory. The Migratory Shorebirds' Action Plan - *Plano de Ação de Aves Limícolas Migratórias*, or PAN Limícolas in portuguese - also recognizes the importance of Lagoa do Peixe for trans-hemispheric shorebirds and highlights it as a stopover for migratory birds (Icmbio 2016).

The National Park has a fluvial or lacustrine inflow with continental and marine waters (Fig. 2f). Due to its connection with the sea, marine waters enter the lagoon creating marshes that provide high productivity of organisms (Andrade *et al*. 2003), which becomes attractive to birds, especially migratory shorebirds. (Frazier 1996, Bencke *et al*. 2006).

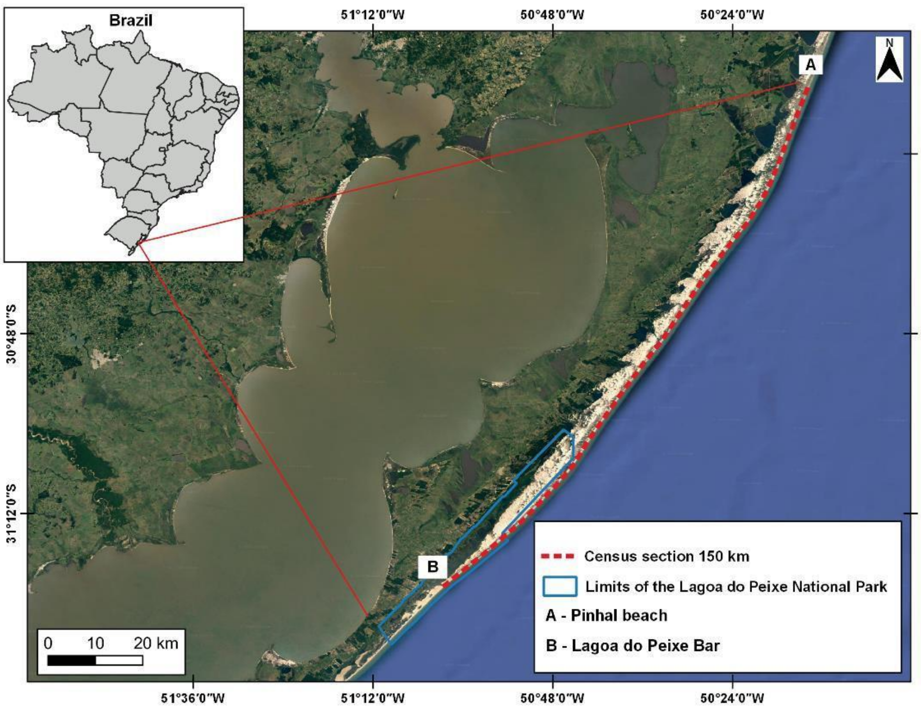



**Fig. 1** - Study area: the dotted line indicates the 150km stretch covered by the middle coast of Rio Grande do Sul, from Pinhal beach (A) to Lagoa do Peixe Bar (B) and the red polygon indicates the limits of Lagoa do Peixe National Park.

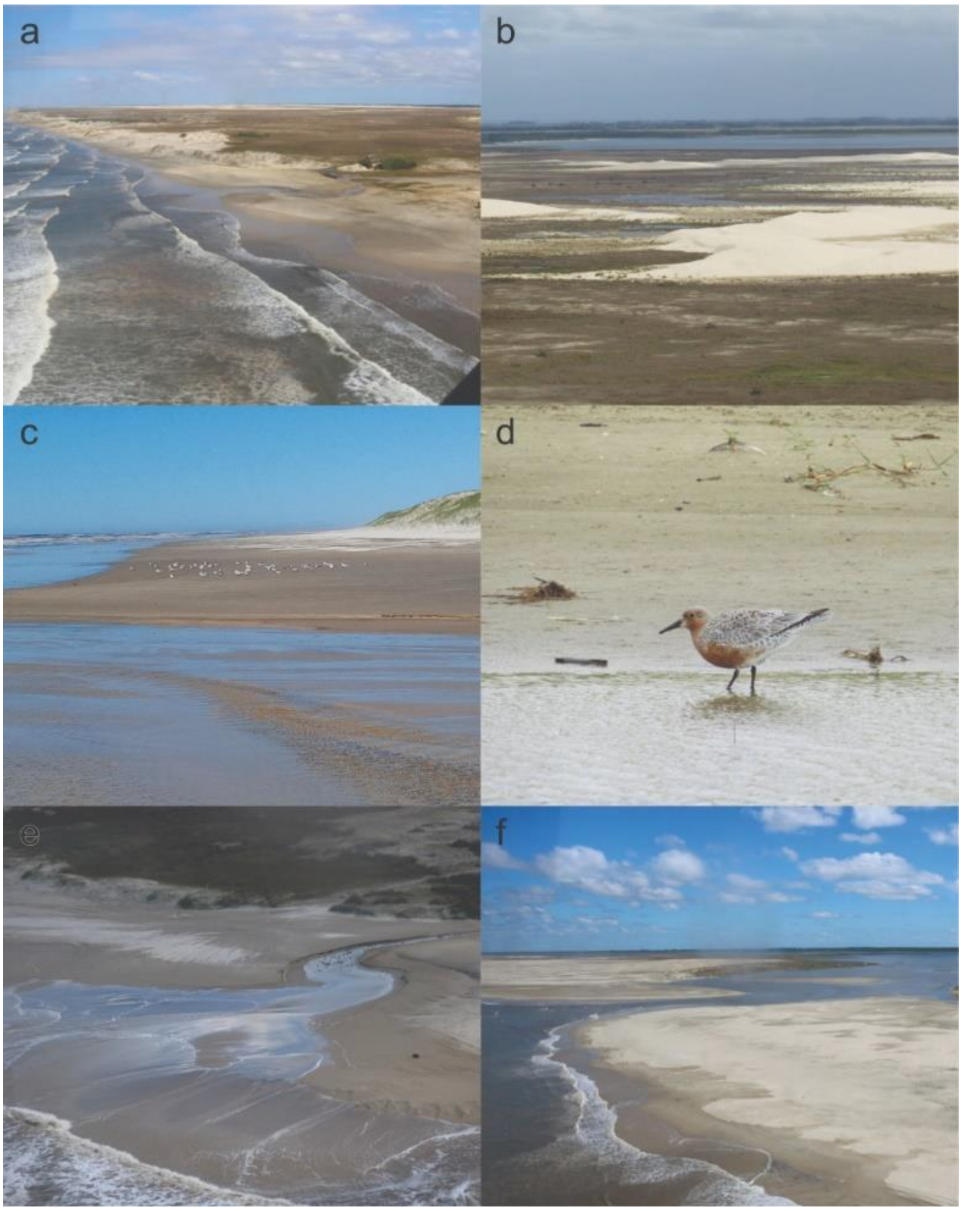



**Fig. 2** - Environments of the study area: **a)** Front dunes, dissipative beach, **b)** Migratory dunes and lagoons, **c)** Dissipative beach with fine sand, **d)** Streams , **e)** Streams and recessed stream mouth, **f)** Inlet bar of Lagoa do Peixe.

***Calidris canutus rufa* census**

We surveyed a 150 km stretch, from Pinhal beach to inlet bar of Lagoa do Peixe, at the middle coast along the beach using a motor vehicle at an average speed of 30 km/h. During the route, all flocks of *C. c. rufa* seen were counted and the coordinates of the occurrence sites were recorded with the aid of GPS. Censuses were conducted during the migratory periods to the south and north, between the years 2019 to 2020. During the northward migration, three censuses were carried out, two in April 2019 and one in April 2020. During the southward migration three censuses were also carried out, one in October 2019, another in October 2020, and the third in November

2020. The censuses started at 8:00 AM. and ended at 5:00 PM. the time needed to cover the 150 km of the study area, including the stops for counting and observations of the flocks. All censuses were carried out on good weather days, with a temperature of 20ºC to 25ºC, northwest wind with a speed between 14-20 km/hr and the tidal amplitude observed on the days of the censuses were on average 0.4-0.8 m. The average annual temperature of the census area between the years 2019 to 2020 was 21.4ºC, according to data obtained from INMEP (Inmep, 2021) and the average precipitation for the year 2019 was 1,608 mm and for the year 2020, it was 2,488 mm, according to precipitation data collected on the Giovanni website (Acker & Leptoukh 2007).

***Sampling of environmental variables***

In order to investigate the relationship between the distribution of flocks and characteristics of the environment, data were collected on the presence of lagoons, stream mouths, wetlands, and urbanized areas. Water and wetland variables were obtained from the hydrography database of the Rio Grande do Sul Environment and Infrastructure State Department (Sema, 2020). Urban areas were identified in the Google Earth software and polygons covering the area of each municipality were created and georeferenced in the ArcGis software with the Measure tool.

The locations of the flocks were georeferenced and the hydrography shapefiles and urban areas were plotted in the ArcGis software (fig.3). Using the Measure tool, the following measurements were then collected: total area of each lagoon (km²), the total area of each wetland area (km²) and each urban area (km²), distance between each flock and the nearest lagoon (km), the distance between each flock and the nearest wetland (km), the distance between each flock and the nearest urban area (km), distance from the closest flock on the same day census (km), in the position of each flock the width of the beach - the distance between the sweep of the water line to the barrier of the dunes - was also measured, as shown in the diagram (Fig. 3). The width of the beach was measured using the measurement tool and the Google Earth@ satellite image (Google, 2020).

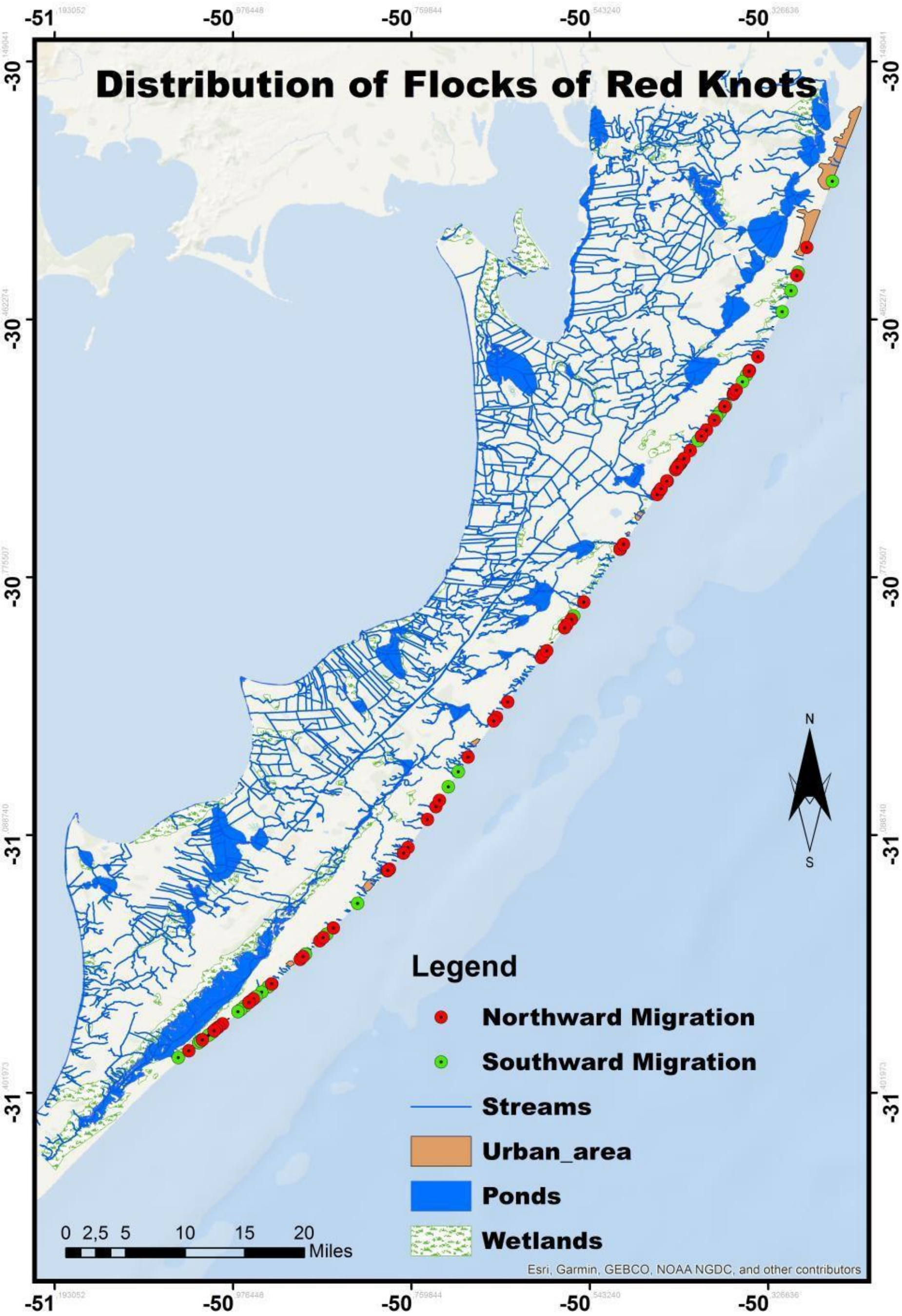



**Fig. 3** - Distribution of flocks of Red Knots during Northward migration (red dots) and Southward migration (green dots), and environmental variables along a 150 km stretch of beach between Pinhal beach and Inlet bar of Lagoa do Peixe.

.

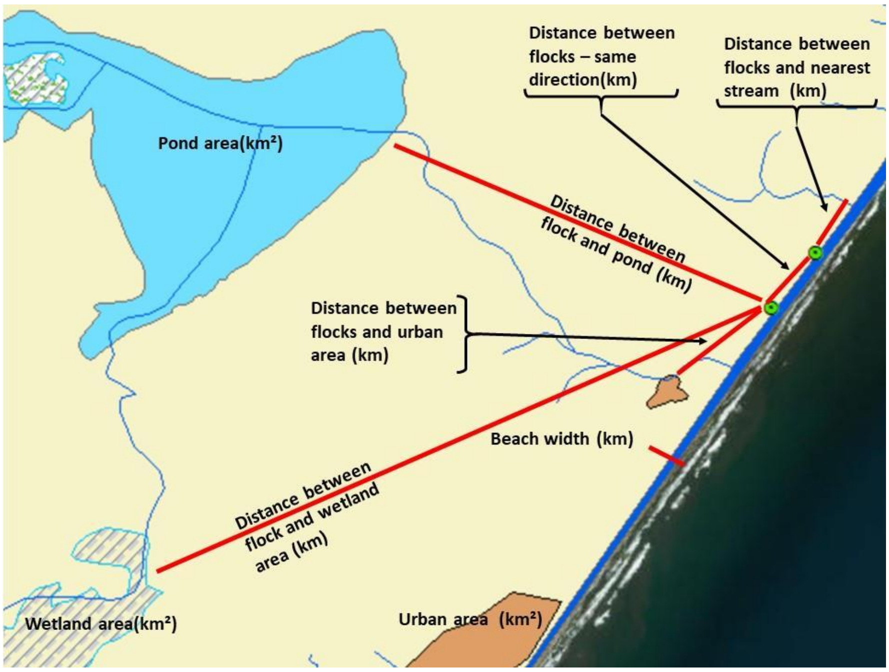



**Fig. 4** - Scheme of metrics in relation to the characteristics of the environment, measured at the places where the Red Knots flocks occur along a 150 km stretch of beach between Balneário Pinhal and Lagoa do Peixe inlet bar.

### *Analytical procedures*

*Abundance and distances between flocks*

In order to test whether the Red Knots flocks varied in the size (i.e., abundance of individuals) and showed different patterns of spatial segregation between the southward and northward migrations, a repeated measure hypothesis test was used. The Shapiro-Wilk test identified the data distribution as non-parametric (W = 0.49327, p = 2.2 e-16) and the homoscedasticity test indicated that the variances are not homogeneous (F = 4,774, p = 6,543e-07). Therefore, a non-parametric and paired Wilcoxon test was applied, using the "*wilcox.test*" function of the "*rstatix*" package in software R version 4.0.0 (R Core Team, 2020). The flock size and the distance between flocks during the southward and northward migration were compared.

*Flock clusters and environmental similarity*

In order to investigate whether the Red Knots showed a preference for specific areas of the coast of the middle coast and the Lagoa do Peixe National being posteriorly grouped according to environmental similarities among them. At a glance, all those flocks may be considered a single large group occupying so extensive geographical area. However, some of them (subgroups) may be more associated to particular variables while others may be more influenced by other ones. Therefore, we employed a hierarchical clustering analysis to determine a number of potential inner groups (flocks) that could be distinguished according to environmental similarity found at those places. As such, As such, the habitat was defined according to the 10 metrics related to environmental variables and limits (distances between flocks, flock size). The metrics were standardized using the *rrank* function in the *'vegan'* package (Oksanen *et al*. 2018). Those standardized metrics used to perform PCAs were also used to carry out such a clustering analysis. Subsequently, a partition of the groups migrating to the north and south was performed using the *hcut* clustering method relating to the metrics through the fviz_nbclust function of the 'factoextra' package (Kassambara & Mundt 2017). This method computes hierarchical clustering and cut the tree in k pre-specified clusters. Through this function, it was possible to estimate the number of those more similar groups of Red Knots using the GAP statistical method that compares the dispersion within the groups for a zero reference distribution (Kassambara & Mundt 2017) after 999 permutations. Since exploratory attempts using different number of arbitrary groups (*n* = 10, *n* = 15, *n* = 20) outcome less than five clusters, we used that last value (*n* = 5) as a reference estimate for our analysis.

To help in the visualization of the detected groups, heatmaps were created using the *HeatmapAnnotation* function (metric vatiation in boxplot) followed by Heatmap, both from the '*ComplexHeatmap*' package (Gu *et al*. 2016). To generate the heatmaps, the standardized matrix of environmental variables was transformed into a distance matrix calculated by Pearson's correlation and the clustering method defined as average, *i.e*. UPGMA (unweighted pair group method with arithmetic mean). Finally, the clusters were visually represented with a Principal Component Analysis (PCA) and a dendrogram, performed using the *hcut* and *fviz_dend* functions of the 'factoextra' package (Kassambara & Mundt 2017).

*Influence of metrics and environmental variables*

To assess whether the metrics and environmental variables influenced the distribution of flocks in the study area, a multivariate approach was used through a Principal Component Analysis (PCA). The PCA captures the amount of variation explained by the variables, reducing the dimensionality, transforming them into "new" variables (orthogonal axes or main components) that contain much of the information in the raw data (Paliy & Shankar 2016). Two ordinations were carried out, one for the northward migration and the other for the southward migration.

Considering that the selected variables were in different units of measure, they were standardized before conducting the PCAs transforming the data matrix into a correlation matrix. For this, the function prcomp (scale = TRUE, center = TRUE) was used in the R environment (Ihaka & Gentleman 1996, R Core Team 2020) and plotted using the function fviz_pca_biplot from the 'factoextra' package (Kassambara & Mundt 2017). In the results of the PCAs, the highest positive or negative scores represented by the first two main components (PC1, PC2) indicate the importance (correlation) of the variables along these axes (gradients) facilitating the interpretation (Paliy & Shankar 2016).

*Correspondence between orders of the environmental gradient*

The two orders generated by the PCA (previous section) were compared after transforming the scores of PC1 and PC2 into a Euclidean distance matrix using the *'vegan'* package function (Oksanen *et al*. 2018). Then, the congruence between the target matrix (PCA northward migration) was evaluated against the rotational matrix (PCA southward migration) using the *Procrustes* superimposition method (Gower 1971). This analysis is an orthogonal mapping of least squares, useful to compare two multivariate datasets where the ordering is scaled and rotated to find an optimal superimposition that maximizes the fit of the model (Gower 1971, Peres-Neto & Jackson 2001), taking into consideration here the environmental variables. The *Procrustes* Analysis has as a metric that indicates the degree of agreement between two configurations, M2 ranging from 0 (perfect congruence) to 1 (absence of congruence) (Gower 1971, Peres-Neto & Jackson 2001). This analysis also proves the degree of correlation between both orders.

The congruence test between the PCA orders was analyzed using the *procrustes* function that generated the model and, later, evaluated after 999 permutations with the *Procrustes* randomization test, *i.e.* PROTEST (Jackson 1995) using the *protest* function, both of the package ' *vegan* '(Oksanen *et al*. 2018).

**RESULTS**

In total, 42 flocks were recorded with an average of 26 ± 55 individuals during the southward migration and 63 flocks with 74 ± 119 individuals during the northward migration (Fig. 5). The highest count recorded during the northward migration was on 21 Apr 2020, with 2,812 individuals distributed over 150 km in 16 flocks (175 ± 201 individuals) (Fig. 6). The lowest count during the northward migration was on 29 Apr 2019, with 880 individuals distributed in 16 flocks with an average of 40 ± 40 individuals. During the southward migration, the highest abundance was registered on 11 Sep 2020, with 1,122 individuals, 24 flocks were recorded, composed on average of 47 ± 67 individuals. The lowest count was in the 8 Nov 2019 census, with 73 individuals distributed in 7 groups with an average of 10 ± 12 individuals.

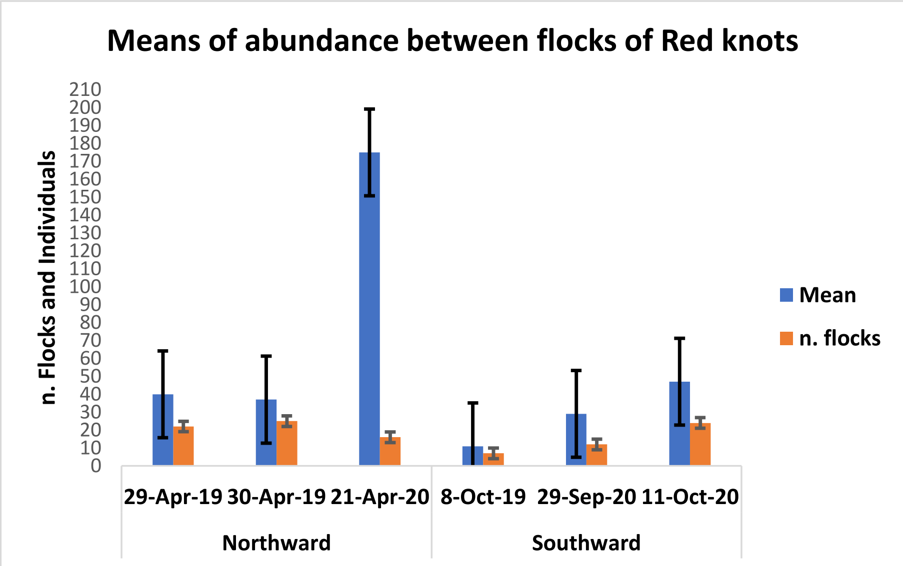



**Fig. 5** -Flock size and number of flocks recorded during the southward and northward migration in the Middle coast of Rio Grande do Sul, The black bar indicates the SE.

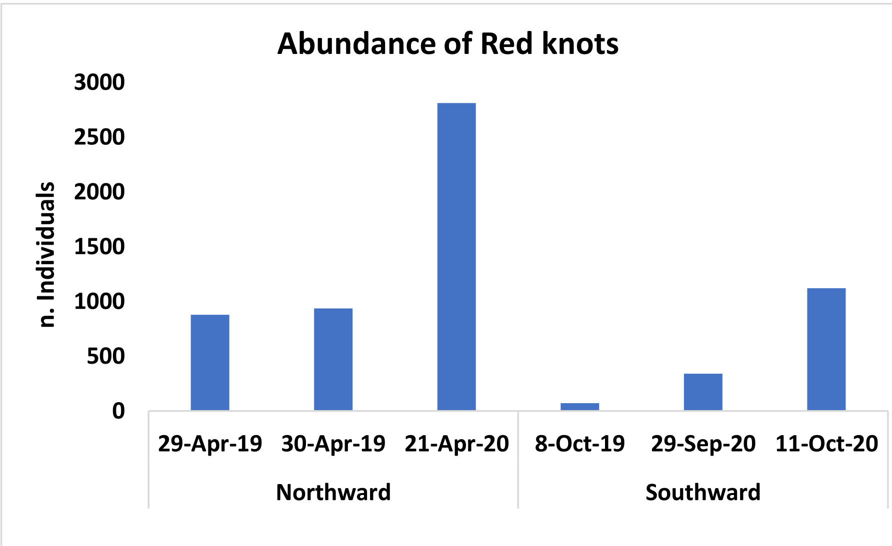



Fig. 06 - Total abundance of Red Knots during their passage through the middle coast of Rio Grande do Sul during the northward and southward migration.

### *Abundance and distance between flocks*

The flocks size of the Red Knots was significantly larger during the northward than the southward migration (Wilcoxon test, W = 1823.5, p = 0.001068). On Average during the northward migration (i.e. during the month of April), 175 individuals were registered per flock, with one flock reaching 800 individuals (Fig. 7a). In the southward migration (during the month of September), on the other hand, the flocks on average were composed of 60 individuals, reaching a maximum of 100 individuals per flock (Fig. 7a).

The distance between the flocks was greater during the southward migration (Wilcoxon test, W = 995.5, p = 0.03243). During the southward migration the flocks maintained a distance of 2 to 10 km (Fig. 7b), while in the northward migration the distances between flocks did not exceed 4 km (Fig. 7b).

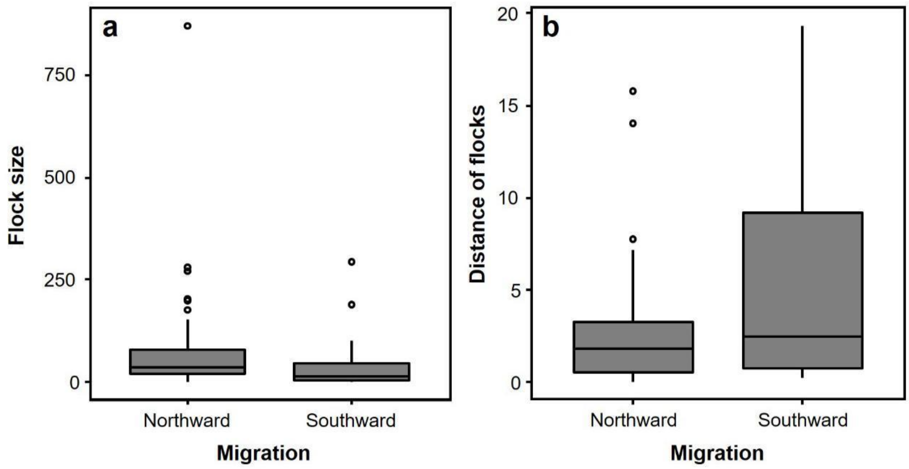



**Fig. 7** - a) Variation in the flock size of Red Knots between northward and southward migration, b) Variation in the distance between flocks of Red Knots between northward and southward migration. Box plot: the box = 1st and 3rd quartiles, whiskers = the min and max range of variation, median (white line) = 2nd quartile, points = outliers.

***Flock clusters and environmental similarity***

Through the hierarchical clustering method, during a northward migration, the formation of two clusters of flocks with respective environmental preferences was detected (Fig. 8a,b). Despite a smaller number of flocks (42 compared to 63 from the northward migration), during the southward migration, four clusters were identified (Fig. 8a,b, inner plots).

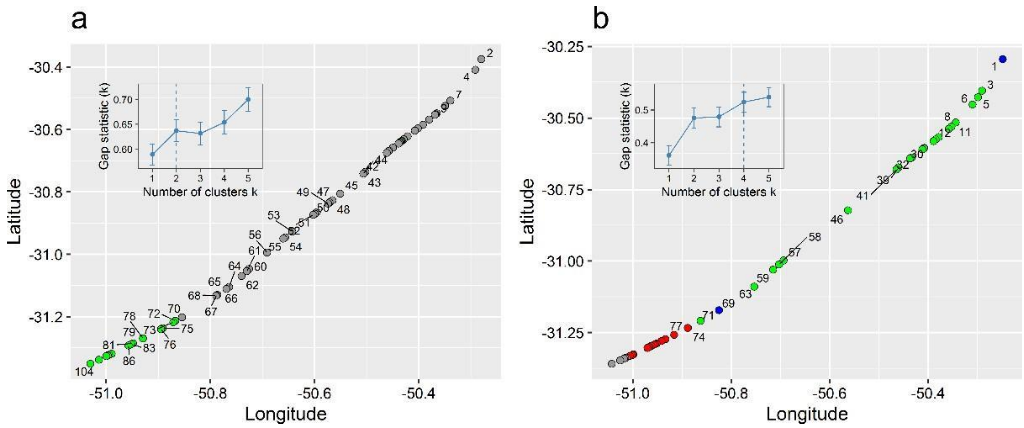



**Fig. 8** - a) Geographic distribution of flocks of Red Knots northward migration, clusters separated by different colors (green and gray), subplot indicates the optimal number of groups detected by the hierarchical clustering method. b) Geographic distribution of flocks of Red Knots Southward migration, clusters separated by different colors (gray, red, green and blue), subplot shows the optimal number of groups detected by the hierarchical clustering method. In inner plots, bars show the mean ± 1 S.E. in gap statistic.

Therefore, while a priori we could have a single large group of red knots, the hierarchical clustering analysis found two (northward) and four (southward) subgroups. Thus, regarding flocks migrating to the north, it is possible to infer that the size of the wetlands and the size of the pond area had a very similar importance to each other. Consequently, they seem to have collaborated to segregate a smaller cluster of flocks of Red Knots, whose presence was related to a higher value of these variables as shown in lower subgroup in heatmap (Fig. 9a). In addition, two other variables, distance from ponds and distances from wetlands, also seem to have played a complementary role in structuring the smallest cluster of Red Knots detected, separating it from the other larger cluster (Fig. 9a).

According to the two inferred clusters (Fig. 9a), it is observed that, during northward migration, flocks further south according to the geographical distribution (Fig. 8a) were influenced by different environmental variables (Fig. 9a) as compared to the northernmost cluster. In a multidimensional representation, it is possible to clearly identify that the clusters formed are quite distinct from each other (Fig. 9b). Finally, on the diagram (Fig. 9c), it is more detailed demonstrated how similar (internally) the Red Knots flocks were.

As for the Red Knots flocks migrating south, it is observed that the largest cluster detected appears to have more values of the distance from the ponds and similarly lower values of wetland and pond areas (upper group in Fig. 10a). On contrary, the second-largest cluster had lower values of these environmental variables, but higher ones with two others, wet area and pond area (middle group in Fig. 10a). There was also the formation of a third cluster containing five flocks being influenced in a very similar way to the second-largest inferred cluster, but including larger values of the drainage distance and distance from the urban area (Fig. 10a). Finally, it was possible to detect that there was the formation of a cluster containing only two flocks that had higher values of the distance of the nearest flock, size of the urban area, and the width of the beach. Nevertheless, such a group had low values of the distance of the drains and distance of the urban areas (lower group in Fig. 10a).

Three out of four clusters detected in the southward migration seem to exhibit a similar pattern from the extreme north to the extreme south of the distribution area of these birds during the migratory process in Rio Grande do Sul (Fig. 10a,c) (green = extreme north, red = median, gray = extreme south). However, one of the clusters generated was based on the similarity found by the presence of these birds in locations whose similarities with the environmental variables were very alike to each other (Fig. 10a,c) (in blue).

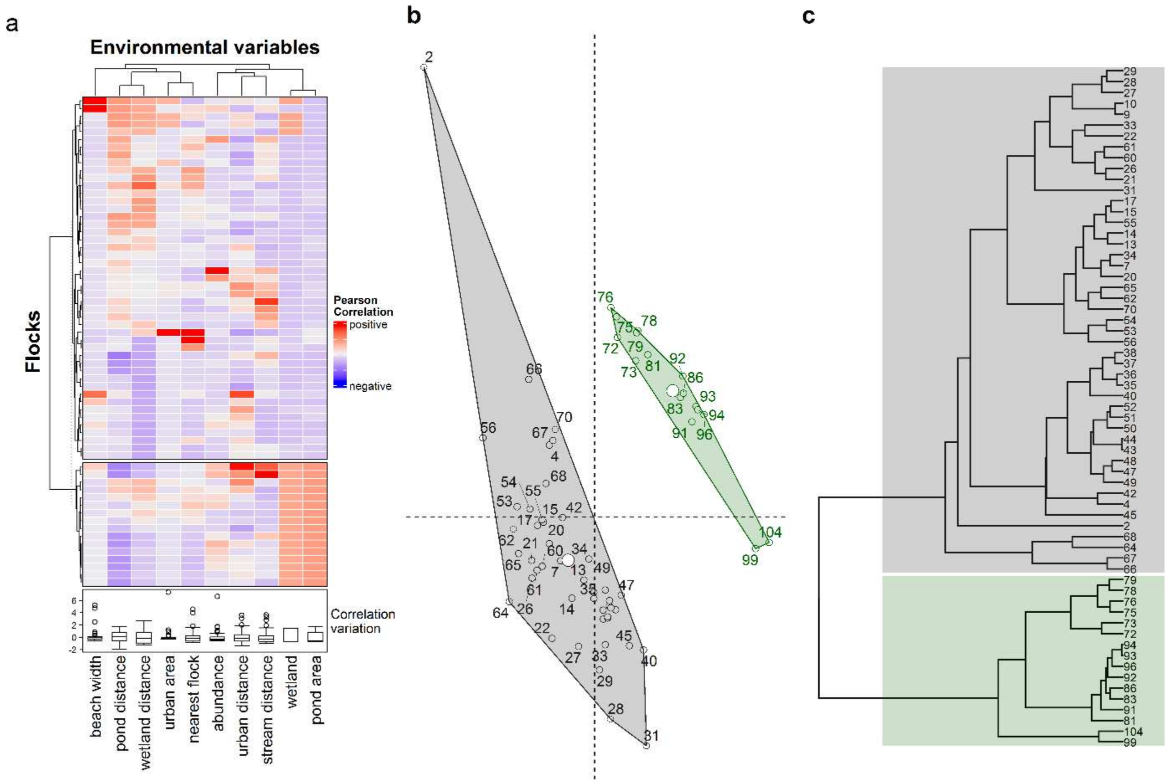



**Fig. 9 - Result of the *kmeans* partition method detecting the optimal number of clusters of flocks of Red Knots migrating to the North. (a)** Heatmap showing a correlation of 10l variables with flocks of Red Knots migrating to the North. Notes: UPGMA clustering method, distance matrix based on Pearson's correlation, boxplots exhibit the variation found in Pearson's correlation across 63 flocks of Red Knots. Additionally, a gap is shown, separating the heatmap into two distinct groups. Therefore, positive correlations can be interpreted as higher values (more red) and negative correlations (more blue) as lower values on the target variable shown in the heat map. **(b)** Clusters detected and represented in a Principal Component Analysis. Notes: clusters surrounded by *convexhull*, white dot shows the centroid, dots and numbers show the identification of

the Red Knot flocks. **(c)** Dendrogram showing the similarity between flocks of Red Knots and the two clusters detected by the *kmeans* method.

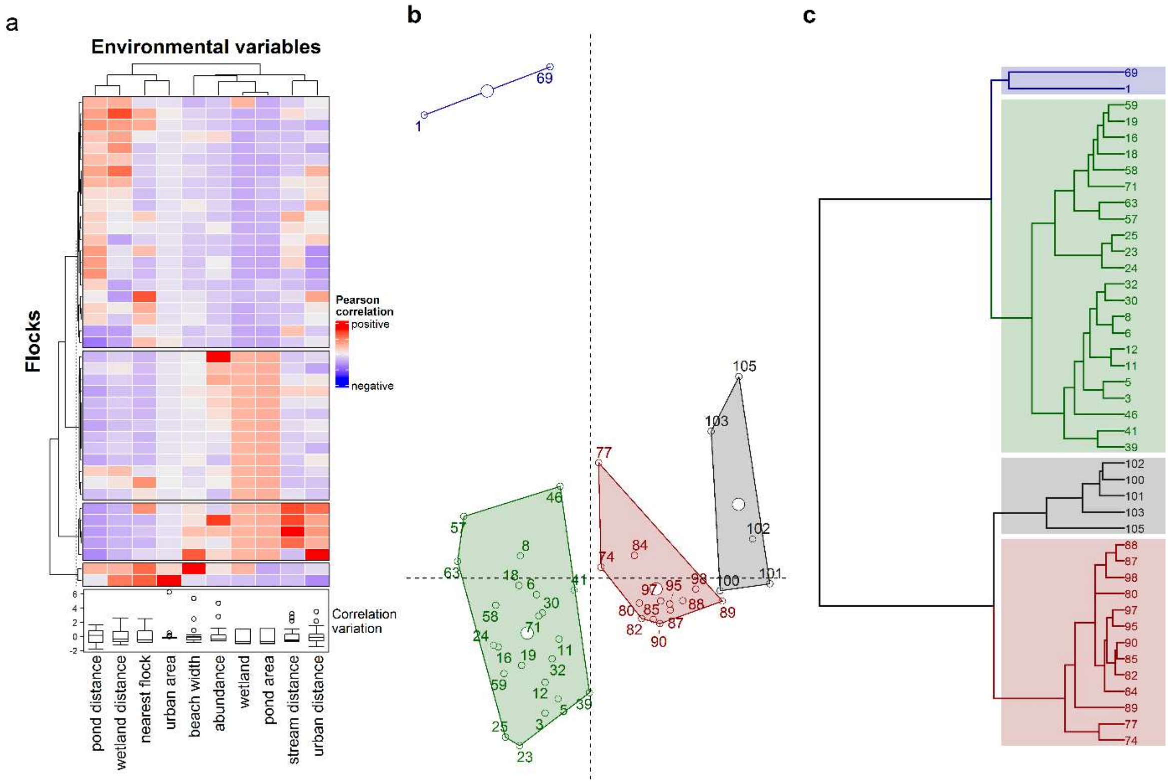



**Fig. 10 - Three distinct graphs showing the results of the *kmeans* partition method by detecting the optimal amount of Red Knot flocks migrating to the south. (a)** Heatmap showing a correlation of 10 environmental variables with flocks of Red Knots migrating to the south. Notes: UPGMA clustering method, distance matrix based on Pearson's correlation, boxplots exhibit the variation found in Pearson's correlation across 42 flocks of Red Knots. Additionally, a gap is shown, separating the heatmap into four distinct groups. . Therefore, positive correlations can be interpreted as higher values (more red) and negative correlations (more blue) as lower values on the target variable shown in the heat map. **(b)** Clusters detected and represented in a Principal Component Analysis. Notes: clusters surrounded by *convexhull*, white dot exhibit centroid, dots and numbers show the identification of the Red Knot flocks. **(c)** Dendrogram showing the similarity between flocks of Red Knots and the four clusters detected by the *kmeans* method.

### *Importance of environmental variables on the occurrence points of Red Knots flocks along a multidimensional gradient*

The Principal Component Analysis explained 43% of the data variation for northward migration. pond distance, pond area, wet area, and wet area distance had, respectively, a strong relationship with the pattern observed along axis 1 (Fig. 11a, Table 1), which was responsible for 25,7% of the data variation. On the other hand, wet area and pond area, again, as well as the urban area, distance from the nearest flock and drainage distance were, respectively, important to explain the pattern observed in axis 2 of the PCA (Fig. 11a, Table 1), responsible for 17,3% of the data variation. The variable that seems to least contribute to and, therefore, to have no explanation for the structure generated in the PCA was the width of the beach, suggesting that it can be neglected by the birds when selecting places to settle during their passage in Rio Grande do Sul during the northward migration (Fig. 11a, Table 1).

On the other hand, the second Principal Component Analysis explained 52.8% of the data variation for the southward migration. Distance from the pond, area from the pond, wet area, and distance from the wet area were again and respectively important to generate the pattern observed along axis 1 (PC1 = 37.3%)(Fig. 10B, Table 1). However, to explain the pattern observed in axis 2 of the PCA (PC2 = 15.5%), variables such as urban area, distance from the nearest flock, beach width, stream distance were, respectively, important (Fig. 11b, Table 1). This last variable had a negligible contribution to order the sampling points of the birds during the northward migration. However, this variable seems to assume greater relevance for birds during their passage migrating to the south (Fig. 11a-b, Table 1).

It is worthwhile to mention that depending on the variable observed along PC1 and PC2, the vector sign of relationships may be positive or negative. Therefore, it suggests that some flocks were more related to high or low values of the variables evaluated here as the gradients (PC1, PC2) move towards left-right or up-down directions.

| **Variables** | **Northward** | | **Southward** | |
|---|---|---|---|---|
| | **PC1** | **PC2** | **PC1** | **PC2** |
| abundance | 0.17 | -0.15 | 0.30 | 0.02 |
| stream distance | 0.22 | -0.34 | 0.25 | -0.03 |
| urban distance | 0.31 | -0.14 | 0.27 | 0.14 |
| urban area | -0.25 | 0.39 | -0.16 | 0.49 |
| pond area | 0.43 | 0.48 | 0.45 | 0.05 |
| pond distance | -0.45 | -0.09 | -0.42 | -0.09 |
| wetland area | 0.35 | 0.51 | 0.41 | 0.23 |
| wetland distance | -0.36 | 0.23 | -0.33 | 0.22 |
| beach width | -0.02 | -0.08 | 0.13 | 0.55 |
| nearest flock | -0.31 | 0.33 | -0.21 | 0.55 |

**Table 1.** Scores of principal component analysis based on environmental variables sampled along the distribution area of flocks of Red Knots at Pinhal Beach to Lagoa do Peixe inlet bar, Rio Grande do Sul.

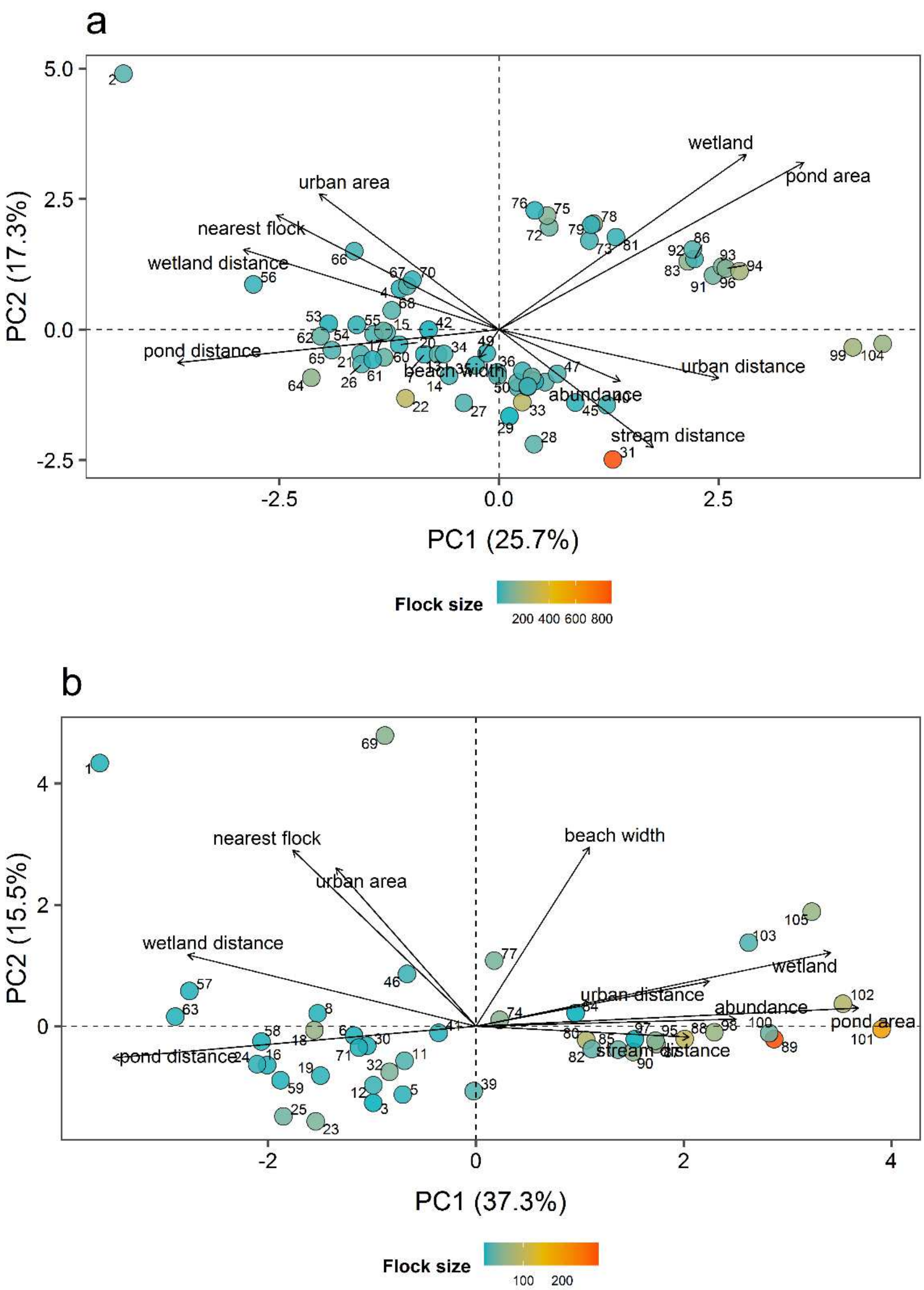



**Fig. 11 - Principal Component Analysis. (a)** Pattern observed for flocks of Red Knots during their migratory passage through Rio Grande do Sul towards the north. **(b)** Pattern observed for flocks of Red Knots during their migratory passage through Rio Grande do Sul towards the south. Note: the main numbers to the points represent the identification of the cataloged flocks, while, the color scale represents the amount of preference (low = blue, median = yellow, high = orange) registered per group, when applicable.

***Congruence between ordering points to a similar pattern of related environmental variables during the northward or southward migration***

The results of the *Procrustes* superimposition analysis comparing the two configurations generated from the scores of the PCAs, northward migration vs. southward migration, indicate that there was a moderate, but meaningful agreement between them (*Procrustes* M2 = 0.33, correlation = 0.81, $p$ = 0.001). Overall, it demonstrates that those environmental variables better explaining the observed pattern in both PCAs had a similar behavior each other. As such, they respond equally on how or whether the habitats selected by different groups of birds as migrating north- or southward were under the same environmental pressure (Fig. 11a,b).

Hence, the contribution of these variables to the pattern in the distribution of flocks and habitat use by Red Knots along the 150 km stretch of the medium coast of Rio Grande do Sul tends to be similar, although the degree of importance may increase or decrease, depending on the configuration.

## DISCUSSION

The Red Knots carry out long-distance migrations and need areas with particular environmental conditions to eat and rest during this journey. This study highlights the importance of the coastal shore of the State of Rio Grande do Sul as a stopover for the species and demonstrates that the birds present different patterns of distribution and spatial segregation in the region and vary in the flock size and abundance of individuals between the northward and southward migrations. Variables related to the characteristics of the environment, such as area and distance from ponds, wetland area and distance, and streams distance, were important to explain the presence and habitat use of Red Knots in the study area, from Pinhal Beach to Lagoa do Peixe inlet bar.

The 2,812 individuals sighted on a single day during the northward migration, represents approximately19% of the migrant population to South America, estimated at 15,000 individuals (Andres *et al*. 2012). Counts in the same area between the years 2007 and 2009, during the months of April and September, did not exceed 1,670 individuals (Scherer & Petry, 2012). However, it is important to note that the study area represents only 24% of the total length of the Rio Grande do Sul coastline that Knot can use (Scherer & Petry, 2012, Bencke *et al*. 2006, Aldabe *et al.* 2015). Thus, the population that passes through the south of Brazil, must represent a greater number of birds than those registered, also this not include turnover.

During migration processes, birds are affected by, in addition to chronological restrictions, several physiological, food, and disturbance limitations (Gonzáles *et al*. 2006, Atkinson *et al*, 2007, Aldabe *et al*. 2015). The difference in the flock size and distribution of flocks in each cluster may reflect the different restrictions that occur in each migration.

In the northward migration the migration window is more compressed in time. There is a greater urgency to keep moving north to maintain a set time table and reach the reproduction areas at the right time for reproduction (Morrison *et al*. 2004, Dey *et al*. 2011). Therefore, birds need to acquire enough energy to reach the next stopover in a short time window (Gonzáles *et al.* 1996, Baker *et al*. 2004, Niles *et al*, 2008). Large flocks favor the use of social cues for the efficient location of prey since the influence of dominant individuals is reduced (Bijilevel *et al*. 2015). Flocks with an abundance of individuals also decrease the constant need for individual vigilance, which can increase the rate of food intake (Burger *et al*, 2007, Lillyman *et al*. 2016). On the other hand, very large flocks increase intraspecific competition, which could lead to local depletion of prey (Bijilevel *et al*. 2012). Thus, the sustenance of a large flock is related to a great availability of prey (Burger *et al*. 2007), a case of the coast of Rio Grande do Sul that has a high abundance of marine invertebrates (Lara- Resende & Leeuwenberg 1987, Gianuca 1998, Farias *et al*, 2018). Hence, the maintenance of large flocks during the northward migration seems to be a strategy to obtain more prey during the small time window such that the Knots can satisfy their need to increase intake rates and gain weight quickly (Niles *et al*, 2006, Atkinson, *et al*, 2007).

In the other hand, during the southward migration, the Red Knots have a much greater timespan to migrate because they are not committed to reproduction. The birds then disperse more and explore more environments, staying longer in stopovers (Niles *et al*. 2008). In this way, individuals can form smaller flocks that, despite being under the influence of dominant individuals, are also under less competitive pressure (Bijilevel *et al*. 2015). In addition, in the southward migration, females leave the reproductive colonies a month before males, contributing to a greater dispersion of the population (Leyrer *et al*. 2012). These characteristics may have contributed to the greater distance between flocks, the smallest flock size and lower abundance of individuals observed during the southward migration compared to the northward migration.

Harrington, Antas & Silva 1986, in research carried out in the area, were the first to observe that there were segregations of flocks of Red Knots in specific places on the coast. Interestingly, some flocks were present in places where there wasn't an abundance of prey and absent in places of high prey abundance. The authors suggested that other variables, in addition to prey density, could be regulating the presence of birds in these sectors. In fact, in this study we found that Red Knots flocks were structured in different ways during the northward and southward migration. The spatial patterns were influenced by different variables, although the tests reveal a high similarity between them, indicating that the environment used by birds in the middle coast of the Rio Grande do Sul is quite similar.

During the northward migration, our survey results indicate that the birds segregated into two large clusters containing several flocks each: one concentrated towards the northern extent of the study area and the other to the south. The distribution of the segregated flocks in the southern portion had a correlation with the area of the ponds and wetland with values higher than with the other variables, demonstrating a strong influence of these variables in the separation of these flocks further south. Lagoa do Peixe and the extensive adjacent wetlands that are in this sector of the study area are located very close to the beach areas that the Red Knots grouped in the southern portion were using for foraging. The salt marshes formed at the edge of Lagoa do Peixe and the sand and silt banks formed by the flow of the tide that enters the lagoon are used as resting areas by Red Knots and several other coastal birds (Fedrizzi, 2008, Scherer & Petry, 2012, Petry *et al*. 2013). When the tide rises and the beach is unavailable for foraging, the Red Knots move to the wet areas, which due to their proximity reduce the energy expenditure used for displacement (Matinez-Curci *et al*. 2020). This behavior is also mentioned by Cohen *et al*. 2009, who supports the idea that the Red Knots can select foraging environments that are up to 4 km away from their resting areas. These particularities of the southernmost environment of the study area may justify the concentration of flocks in this area.

On the other hand, the distribution of the cluster of birds in the northern portion had a high correlation with the variables area of ponds and wetlands and low when related to the distance from ponds and wetlands. In the northern portion of the census area, the ponds are smaller and there are few wetland areas, which may not be attractive to the Knots - in addition to these being areas with greater anthropic pressure, and birds having the ability to avoid areas with a lot of disturbance or that represent a high risk of predation (Burger *et al.* 2013). Otherwise, that the high correlation value of the presence of flocks with the distance from the pond and wetlands, although mathematically possible, is not factually reliable. If birds are not using these inlands, the high correlation found in the northern section is only a function of distance.

Therefore, there must be other variables, which were not the object of this study, that could better explain the cluster of these Red Knots flocks in this stretch of coast. One of the hypotheses is that, with the proximity of the beginning of migration, the birds opt for short displacements further north, until they start a long migration flight along the Atlantic coast. However, it is important to note that in this area, close to the Farol da Solidão (30º42´3.59´´S, 50º 29´1.95´´W), in the last monitoring, large flocks of Red Knots are invariably found (unpublished data), which may be linked to birds' fidelity to foraging areas too (Leyer *et al*. 2006, Newstead *et al*. 2013).

During the southward migration, it was detected the formation of four optimal groups of Red Knots flocks along the study area, and it was possible to observe that the flocks were more distributed than in the model verified during the northward. However, it was possible to identify a group that was high influence by drainage and urban areas. It was expected that in areas where there were drainage channels, there might be a greater presence of flocks of birds close to them. In these places there is a retreat from the frontal dunes, creating a different environment that works as habitats for resting due to the widening of the beach and protection from the winds by the dunes, in addition to the formation of small pools of fresh water, which serve as a place for the care of plumage. This result is also corroborated by the visual comparison of the map (Fig. 3) where it is possible to see that the flocks are usually at the exit of these streams channels from wetland areas and ponds. Previous study showed that Red Knots, in Hudson Bay - Canada, frequent freshwater streams and lagoons near the mouth of the Hudson River (Mckellar *et al*. 2015), Although the primary habitat of the Red Knots' consists of the intertidal zones in the swash zone, other coastal features such as estuaries, bays, waterlogged areas, and freshwater also appear important (Burger *et al*. 2015). In the Valdes Peninsula, in Argentina, it has been shown that birds use restinga areas as a resting place and as foraging areas (Musmeci *et al*. 2015). Other studies have also revealed the use of lagoons with salt marshes and wetlands as alternative sites for foraging (Farmer *et al*. 2004, Bishop *et al*. 2016, Oudman et al. 2018). A study carried out in the Lomas Bay - Chile with an analysis of stable isotopes in wintering Red Knots' feathers indicated the influence of fresh water on the diet of those individuals (Atkinson *et al*. 2005), suggesting the use of these environments by birds. In Hudson Bay - Canada, Red Knots frequent freshwater streams and lagoons near the mouth of the Hudson River (Mckellar *et al*. 2015).

In view of this, it is important to identify the characteristics that exist in the environment and to study their influence on habitat use by Red Knots. In our study both by the analysis of hierarchical clusters, as by the sploratory analysis performed by the

PCA, it is possible to identify that some variables play a more important role than others or act together to segregate the Red Knots flocks. In the case of the heatmap clustering analysis, for example, the variables distance and area of lakes and wetlands play similar roles in the segregation of flocks, whereas in PCA the variables that are more representative for the distribution of flocks along the x and y gradient are distance from urban area and drainage channels. It should not be forgotten that Red Knots are passing through and that other dynamic variables such as arrival time, climate change, prey availability, competition and disturbance can exert a strong pressure on the selection of the habitat that the bird will find at the expense of other more ecologically obvious ones. The challenge is to determine which variables are most important and which characteristics in the environment contribute to the bird establishing itself in certain places and not in others, even if only for a short period of time. In the study, for example, we observed that close to larger urban areas, the number of flocks was smaller in both migrations (Fig. 3) and the greater the distance from the urban area, the flock size increases. It is possible that this is because birds avoid areas with more disturbance (Burger et al. 2013). In the case of drainage channels, which were also important, as mentioned above, it can be observed in PCA that along this gradient, the further away from the center, the size of the flocks grows.

On the other hand, beach width was the variable that least influenced the presence of flocks, both in the southward and northward migration. This result is not unexpected as the beach width in the middle of the coast is very consistent, with small changes ranging on average 106 meters along the study area, even considering the error of three to four meters, there was no influence in the test results, not even admitting the influence of the tide amplitude that could affect the width of the beach, or that during the censuses, it did not exceed 0.50 m in the vast majority of the census area, with the exception of Pinhal beach, where it reached 0.80 m, but not high enough to make the area unavailable to birds and interfere with their presence, unlike other occurrence sites - such as Punta Rasa, Argentina, where birds alternate during high tide between beaches muddy and coastal environments (Matinez-Curci et al. 2020) and Calidris canutus canutus in Australia (Roger et al. 2004).

### *Importance for conservation*

The study area from Pinhal beach to Inlet bar of Lagoa do Peixe (see fig. 1) comprises 24% of the entire coastal area of the State of Rio Grande do Sul. Much of this area is exploited by forestry with the cultivation of Pinus sp. (Coelho *et al*. 2019), occupied by the energy industry with the presence of installed wind farms (Verdum *et* *al*. 2020), and used by the general population through leisure and urbanization areas. However, few environmental protection actions, such as establishing protection for coastal ponds, prohibiting the spraying of pesticides in areas that are connected with drainage to coastal ponds, and limiting the planting of exotic vegetation, have been implemented. The Lagoa do Peixe National Park covers only 62 km of the length of the coast, and even this small portion is being neglected and under duress by sectors of the population and by the government agencies that have a duty to protect it.

Across a large part of the area of Rio Grande do Sul beach front used by Red Knots during their migration stopover, we observed a number of sources of disturbance, such vehicular traffic recreational and work vehicles (fishermen), pedestrians, presence of domestic and semi- feral dogs, pollution by anthropogenic debris (Brum *et al*. personal observation). Even within the National Park, managers have struggled to reduce tourist vehicle traffic on the beaches during the months of migration.

The present work, by demonstrating the extensive use of the beaches of the middle coast by the Red Knot, as well as its association with adjacent lagoons, ponds and streams, highlights how the entire coastal land scape is important to knots as vital habitat needed to ensure successful migration. In addition, this work provides support for conservation actions to be adopted at least during migratory periods, which can benefit not only the Red Knot but all birds that use this habitat.

**CONCLUSION**

We observed differences in the structure of *Calidris canutus rufa* flocks, both in southward migration and in northward migration along the coast of Rio Grande do Sul state in southern Brazil. These differences, as our results show, can be explained by the variables of the environment, as well as by the dynamics of the birds' behavior in their stopping areas, by the composition of the flocks, or by the differences in the perception of the environment of each individual, considering their skills in finding food.

Therefore, we corroborate our hypothesis that there is a pattern of bird grouping and habitat use that is different during northward and southward migrations. We were able to conclude that the dynamics of the distribution of flocks can be oriented and explained by environmental variables and variables related to individuals, such as the distance between flocks, in the latter case. It was possible to identify that stream channels, size and distance from urban areas play a fundamental role in the presence of flocks, as well as size and distances of ponds and wetlands that work together to cluster flocks of Red Knots in certain sectors of the beach. In this sense, Lagoa do Peixe, as it is a large coastal lagoon and has many wetland areas, plays a fundamental role in the presence of these birds in the middle coast of Rio Grande do Sul.

### ***Acknowledgment***

This project was partly financed by the National Research and Development Council (CNPQ) (Grant No. 425884 / 2016-3) and by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) through a PROSUC Master's scholarship to Antônio Coimbra de Brum (Finance code 001). The authors would like to thank the Unisinos Graduate Program in Biology, its coordinator Dr. Larissa Rosa de Oliveira and its professors, to colleagues at Unisinos' Laboratory of Ornithology and Marine Animals, To Leonardo Dorneles for his assistance with the tests, Douglas Ribeiro da Silva for his help in preparing the map of the study area and for his partnership in the fields and Antônio Ribeiro da Silva for his help in the field. To the partners of the Bird Watchers Club of Porto Alegre - RS, for their field assistance, in particular, Roberto D´lagnol, Glayson Bencke and Gilberto Müller. Ana Flora for the translation of the manuscript.

# REFERENCES

**Acker J.G. & G. Leptoukh**. 2007. "Online Analysis Enhances Use of NASA Earth Science Data", *Eos, Trans. AGU* 88: 14-17.

**Aldabe, J**. **P. Rocca, P. Gonzalez, D. Caballero-Sadi & A. Baker.** 2015. Migration of endangered Red Knots *Calidris canutus rufa* in Uruguay: important sites, phenology, migratory connectivity and a mass mortality event. *Wader Study 122*: 221-235.

**Andrade, R**. **L.F.Martini & S. P. Dieguez.** 2003. *Parques Nacionais: Brasil*. Empresa das Artes. São Paulo. [in portuguese]

**Andres, B.A**. **P.A. Smith, R.G. Morrison, C.L. Gratto-Trevor, S.C. Brown & C.A. Friis.** 2012. Population estimates of North American shorebirds. *Wader Study Group Bulletin* 119: 178-194.

**Atkinson, P.W**. **A.J. Baker, K.A. Bennet, N.A. Clark, J.A. Clark, K.B. Cole, A. Dekinga, A. Dey, S. Gillings, P.M. Gonzalez, K. Kalasz, C. Minton, J. Newton, T. Piersma, R.A. Robinson & H.P. Sitters.** 2007. Rates of mass gain and energy deposition in red knot on their final spring staging site is both time- and condition-dependent. *J. Appl. Ecol*. 44: 885-895.

**Atkinson, P.W**. **A.J. Baker, R.M. Bevan, N.A. Clark, K.B. Cole, P.M. Gonzalez & R.A. Robinson**. 2005. Unravelling the migration and moult strategies of a long-distance migrant using stable isotopes: Red Knot *Calidris canutus* movements in the Americas. *Ibis*. 147: 738-749.

**Baker, A.J**. **P.M. Gonzalez, T. Piersma, L.J. Niles, I. de Lima S.do Nascimento, P.W. Atkinson, P. Collins, N.A. Clark, C.D.T. Minton, M.K. Peck & G. Aarts**. 2004. Rapid population decline in red knots: fitness consequences of decreased refuelling rates and late arrival in Delaware Bay. *Proceedings of the Royal Society of London* B 271: 875-882.

**Belton, W**. 2000. *Aves do Rio Grande do Sul: Distribuição e biologia*. Universidade do Vale do Rio dos Sinos, São Leopoldo. 98p. [In Portuguese]

**Bencke, G.A**. **G.N. Maurício, P.F. Develey & J.M. Goerck**. 2006. *Áreas Importantes para a Conservação das Aves no Brasil*. Parte I–Estados do Domínio da Mata Atlântica. SAVE Brasil, São Paulo. [In Portuguese]

**Bijleveld, A.I, E.O. Folmer & T. Piersma.** 2012. Experimental evidence for cryptic interference among socially foraging shorebirds. *Behavioral Ecology* 23: 806-814.

**Bijleveld, A.I**. **J.A.Van Gils, J. Jouta & T. Piersma.** 2015. Benefits of foraging in small groups: an experimental study on public information use in red knots *Calidris canutus*. *Behavioural Processes***.** 117: 74-81.

**Bishop, M.A**. **J.B. Buchanan**. **B.J. Mccaffery & J.A. Johnson**. 2016. Spring stopover sites used by the red knot *Calidris canutus roselaari* in Alaska, USA:

Connectivity between the Copper River Delta and the Yukon–Kuskokwim River Delta. *Wader Study* 123: 143-152.

**Brasil. 1986**. *Decreto nº 93.546, de 6 de novembro de 1986*. Planalto, Brasília. Accessed 30 Nov 2019 at: http://www.planalto.gov.br/ccivil_03/decreto/1980-1989/1985-1987/D93546.htm. [In Portuguese]

**Buehler, D.M**. **L. Bugoni, G.M**. **Dorrestein, P.M. González, J**. **Pereira Jr, L**. **Proença, ... & T. Piersma.** 2010. Local mortality events in migrating sandpipers (Calidris) at a staging site in southern Brazil. *Wader Study Group Bulletin* 117: 150-156.

**Burger, J. & J.L. Niles**. 2013. Closure versus voluntary avoidance as a method of protecting migrating shorebirds on beaches in New Jersey. *Wader Study Group Bulletin* 120: 20-25.

**Burger, J**. **L. Niles, C. Jeitner & M. Gochfeld.** 2018. Habitat risk: Use of intertidal flats by foraging red knots (*Calidris canutus rufa*), ruddy turnstones,(*Arenaria interpres*), semipalmated sandpipers (*Calidris pusilla*), and sanderling (*Calidris alba*) on Delaware Bay beaches. *Environmental research* 165: 237-246.

**Burger, J**. **L.J. Niles, R.R. Porter, A.D. Dey, S. Koch & Gordon, C.** 2012. Migration and over-wintering of red knots (*Calidris canutus rufa*) along the Atlantic coast of the United States**.** *The Condor* 114: 302-313.

**Burger, J., M. Gochfeld, L. Niles, C. Powers, K. Brown, j. Clarke, ... & D. Kosson**. 2015. Complexity of bioindicator selection for ecological, human, and cultural health: Chinook salmon and red knot as case studies. *Environmental monitoring and assessment* 187: 102.

**Burger, J., S.a. Carlucci, C.W. Jeitner & L. Niles.** 2007. Habitat choice, disturbance, and management of foraging shorebirds and gulls at a migratory stopover. *Journal of Coastal research* 23: 1159-1166.

**Catry, T., P.M. Lourenço, R.J. Lopes, P. Bocher, C. Carneiro, J.A. Alves, ... & J.P. Granadeiro**. 2016. Use of stable isotope fingerprints to assign wintering origin and trace shorebird movements along the East Atlantic Flyway. *Basic and Applied Ecology* 17: 177-187.

**Coelho-de-Souza, G., J.D. Ramos, A.R. Teixeira, N.M. Schreiber, R.S.P. Mello &** B. **Rocha**. 2019. Governança da política de desenvolvimento territorial no rio grande do sul: dinâmicas no contexto socioambiental dos territórios rurais litoral e campos de cima da serra. *Revista Margens Interdisciplinar* 13: 19. [In Portuguese]

**Cohen, J.B**. **S.M. Karpanty, J.D. Fraser, B.D. Watts & B.R. Truitt.** 2009. Residence probability and population size of Red Knots during spring stopover in the mid-Atlantic region of the United States. *The Journal of Wildlife Management* 73: 939-945.

**Cohen, J.B., S.M. Karpanty & J.D. Fraser. 2010**. Habitat selection and behavior of red knots on the New Jersey Atlantic coast during spring stopover. *The Condor* 112: 655-662.

**Dey, A.D., L.J. Niles, H.P. Sitters, K. Kalasz & R. G. Morrison.** 2011. Update to the status of the Red Knot *Calidris canutus* in the western hemisphere, April 2011. *Western Hemisphere Reserve Network*.

**Faria, F.A., E.F. Albertoni & L. Bugoni.** 2018. Trophic niches and feeding relationships of shorebirds in southern Brazil. *Aquatic Ecology* 52: 281-296.

**Farmer, A., M. Abril, M. Fernández, J. Torres, C. Kester & C. Bern**. 2004. Using stable isotopes to associate migratory shorebirds with their wintering locations in Argentina. *Ornitología Neotropical* 15: 1-8.

**Fedrizzi, C.E.** 2008. *Abundância, distribuição e ecologia de forrageio de ave costeira (*Charadriiformes: Charadrii and Scolopaci*) na Costa do Rio Grande do Sul, Brasil*. Tese de doutorado, defendida na Universidade Federal de Rio Grande, Rio Grande. [In portuguese]

**Fraser, J.D, S.M. Karpanty, J.B. Cohen & B.R. Truitt.** 2013. The Red Knot (*Calidris canutus rufa*) decline in the western hemisphere: is there a lemming connection? Canadian Journal of Zoology 91: 3-16.

**Gianuca, N.** 1998. Ambientes Costeiros e Marinos e sua biota: Invertebrados Bentônicos da Praia. Page 326. *In* U. Seelinger. C. Odebrecht, and J. P. Castello, (editores). Os Ecossistemas Costeiros e Marinhos do Extremo Sul do Brasil. Ecoscientia, Rio Grande. [In Portuguese]

**Gilroy, J.J. J.A. Gill, S.H. Butchart, V.R. Jones & A.M. Franco**. 2016. Migratory diversity predicts population declines in birds. Ecology letters 19: 308-317.

**González, P. M. A.J. Baker & M.E. Echave.** 2006. Fundación Inalafquen. Annual survival of Red Knots (Calidris canutus rufa) using the San Antonio Oeste stopover site is reduced by domino effects involving late arrival and food depletion in Delaware Bay. *El hornero*.

**González, P.M., T. Piersma & Y. Verkuil.** 1996. Food, Feeding, and Refuelling of Red Knots during Northward Migration at San Antonio Oeste, Rio Negro, Argentina (Alimentacion y Reabastecimiento de Playeros Rojizos Calidris canutus rufa Durante su migracion al Norte en San Antonio Oeste, Rio Negro, Argentina). *Journal of Field Ornithology*, 575-591.

**Google Erth**. 2020. Accessed 20 Apr 2020. at: www.googleerth.com.

**Gower, J.C.** .1971. Statistical methods of comparing different multivariate analyses of the same data. *Math Archaeol Hist Sci* 138–149.

**Gu, Z, R. Eils & M. Schlesner**. 2016. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* 32 :2847–2849.

**Harrington, B. A**. **J.M. Hagan & L.E. Leddy.** 1988. Site fidelity and survival differences between two groups of New World Red Knots (*Calidris canutus*). *The Auk* 105: 439-445.

**Harrington, B.A**. **P.T.Z. Antas & F. Silva**. 1986. Northward shorebird migration on the Atlantic coast of southern Brazil. *Vida Silvestre Neotropical* 1: 45-54.

**Harrington, B.A**. **S. Loch, L. Niles & K. Kalasz.** 2010. Red Knots with different wintering destinations: differential use of an autumn stopover area. *Waterbirds* 33: 357-363.

**Harrington, B.A., B. Winn & Brown.** 2007. Molt and body mass of red knots in theeastern united states. *The Wilson Journal of Ornithology*, 119: 35-42.

**Icmbio. 2016**. *Relatório anual de rotas e áreas de concentração de aves migratórias no Brasil.* Cabedelo, PB: CEMAVE/ ICMBio, Brasília. [In Portuguese]

**Inmep - Instituto Nacional de Metereologia**. 2021. *Dados metereológicos*. Accessed at: https://portal.inmet.gov.br/. [In Portuguese]

**Ihaka R & R. Gentleman.** 1996. R: a language for data analysis and graphics. J Comput Graph Stat 5:299–314.

**Jackson, D.A.** 1995. PROTEST: a Procrustean randomization TEST of community environment concordance. *Ecoscience* 2:297–309

**Johnson, O.W**. **L. Fielding, J.W. Fox, R.S. Gold, R.H. Goodwill & P.M. Johnson**. 2011. Tracking the migrations of Pacific Golden-Plovers (*Pluvialis fulva*) between Hawaii and Alaska: New insight on flight performance, breeding ground destinations, and nesting from birds carrying light level geolocators. *Wader Study Group Bull* 118: 26-31.

**Kassambara, A & F. Mundt.** 2017. factoextra: Extract and visualize the results of multivariate data analyses. Accessed 22 Nov 2020 at: https://www.r-project.org/.

**Lara-Resende, S. & F. Leeuwenberg**. 1987. Ecological studies of Lagoa do Peixe. Final Report to WWF-US, *Washington* 1: 52.

**Lathrop, R.G**. **L. NILES, P. SMITH, M. PECK, A. DEY, R. SACATELLI & J. BOGNAR**. 2018. Mapping and modeling the breeding habitat of the Western Atlantic Red Knot (*Calidris canutus rufa*) at local and regional scales. *The Condor: Ornithological Applications* 120: 650-665.

**Leyrer, J. B. Spaans, M. Camara & T. Piersma.** 2006. Small home ranges and high site fidelity in red knots (*Calidris c. canutus*) wintering on the Banc d'Arguin, Mauritania. Journal of Ornithology, 147: 376-384.

**Leyrer, J**. **T. Lok, M. Brugge, A. Dekinga, B. Spaans, J.A. Van Gils, ... & T. Piersma**. 2012. Small-scale demographic structure suggests preemptive behavior in a flocking shorebird. *Behavioral Ecology* 23: 1226-1233.

**Lilleyman, A., D.C. Franklin, J.K. Szabo & M.J. Lawes.** 2016. Behavioural responses of migratory shorebirds to disturbance at a high-tide roost. Emu-Austral Ornithology 116: 111-118.

**Lyons, J.E**. **B. Winn, T. Keyes & K.S. Kalasz.** 2018. Post-breeding migration and connectivity of red knots in the Western Atlantic. *The Journal of Wildlife Management* 82: 383-396.

**Martínez-Curci, N.S**. **J.P. Isacch, V.L. D'amico, P. Rojas & G.J. Castresana.** 2020. To migrate or not: drivers of over-summering in a long-distance migratory shorebird. *Journal of Avian Biology* 51: 10.

**Mckellar, A.E**. **R.K Ross, R.G.Morrison, L.J. Niles, R.R. Porter, J. Burger, ... & P.A. Smith.** 2015. Shorebird use of western Hudson Bay near the Nelson River during migration, with a focus on the Red Knot. *Wader Study* 122: 201-211.

**Morrison, R.I**. **R.R. Kenyon & L.J. Niles**. 2004. Declines in wintering populations of Red Knots in southern South America. *The Condor* 106: 60-70.

**Motta, L.M., E.E.Toldo, L.E. Sá, B. Almeida & J.C. Nunes.** 2015. Sandy sediment budget of the midcoast of Rio Grande do Sul, Brazil. *Journal of Marine Research* 73: 49-69.

**Musmeci, L.R., M.A.Hernández, L.O Bala & J.A. Scolaro.** 2015. Cambios temporales en la dieta del Playero Rojizo (*Calidris canutus rufa*) en un humedal de Península Valdés, Patagonia Argentina. [In Spanish]

**Musmeci, L.R., M.A.Hernández, L.O Bala & J.A. Scolaro.** 2012. Península Valdés, Patagonia: A system of beaches used by Red Knots (*Calidris canutus rufa*) working as a functional unit. *Emu* 112: 357-362.

**Newstead, D.J**. **L.J. Niles, R.R. Porter, A.D. Dey, J. Burger, & O.N. Fitzsimmons**. 2013. Geolocation reveals mid-continent migratory routes and Texas wintering areas of Red Knots *Calidris canutus rufa*. *Wader Study Group Bulletin* 120: 53-59.

**Niles, L.J**. **H.P. Sitters, A.D. Dey, P.W. Atkinson, A.J. Baker, K.A. Bennett, ... & I.L. Serrano.** 2008. Status of the Red Knot (*Calidris canutus rufa*) in the Western Hemisphere. In: *Status of the Red Knot (Calidris Canutus Rufa) in the Western Hemisphere 2008*: 1-185.

**Niles, L.J**. **J. Burger, R.R. Porter, A.D. Dey, C.D. Minton, P.M. González & C. Gordon.** 2010. First results using light level geolocators to track Red Knots in the Western Hemisphere show rapid and long intercontinental flights and new details of migration pathways. *Wader Study Group Bull* 117: 123-130.

**Niles, L.J**. **J. Burger, R.R. Porter, A.D. Dey, S. Koch, B. Harrington, & M. Boarman**. 2012. Migration pathways, migration speeds and non-breeding areas used by northern hemisphere wintering Red Knots *Calidris canutus* of the subspecies *rufa*. *Wader Study Group Bull* 119: 195-203.

**Niles, L.J., H.P Sitters, A.D Dey, A.J Baker, R.I.G. Morrison, K.E. Clark, B.A. Harrington, M.K. Peck, P.M. Gonzalez, P.W. Atkinson, K.A. Bennett, C.D.T. Minton & K.S. Kalasz**. 2006. Uncertainty about the size and status of Red Knot populations in the Americas is a problem for conservation. *Wader Study Group Bull* 109: 39.

**Oksanen, J., F.G. Blanchet & M. Friendly**. 2018. *vegan: Community Ecology Package*. Accessed 21 Nov 2020 at: https://cran.r-project.org.

**Oliveira, A.D**. **A.E.A. Barbosa, A.E.B.A. Sousa, C. Lugarini, D.M. Lima, J.L.X. Nascimento, ... & P.P. Amaral.** 2016. *Relatório anual de rotas e áreas de concentração de aves migratórias no Brasil*. **CEMAVE/ICMBio, Cabedelo**. Brasília. [In Portuguese]

**Oudman, T**. **T. Piersma, M.V.A. Salem, M.E. Feis, A. Dekinga, S. Holthuijsen, ... & A. I. Bijleveld.** 2018. Resource landscapes explain contrasting patterns of aggregation and site fidelity by red knots at two wintering sites. *Movement ecology* 6: 1-12.

**Oudman, T., T. Piersma, M.V.A Salem, M.E. Feis, A. Dekinga, S. Holthuijsen, ... & A.I. Bijleveld**. 2018. Resource landscapes explain contrasting patterns of aggregation and site fidelity by red knots at two wintering sites. *Movement ecology* 6: 1-12.

**Paliy, O. & V. Shankar.** 2016. Application of multivariate statistical techniques in microbial ecology. *Mol Ecol* 25:1032–1057

**Peres-Neto, P.R. & D.A. Jackson**. 2001. How well do multivariate data sets match? The advantages of a procrustean superimposition approach over the Mantel test. *Oecologia* 129:169–178.

**Petry, M.V., A.L.Scherer, J.F.M. Scherer, V.H. Valiati.** 2013. Sexual Dimorphism, Habitat Use and Molt in Wintering Black Skimmers (*Rynchops niger*) in the Lagoa do Peixe, Southern Brazil. Waterbirds 36: 438-447.

**Piersma, T**. 2007. Using the power of comparison to explain habitat use and migration strategies of shorebirds worldwide. *Journal of Ornithology* 148: 45.

**R Core Team**. 2020 R: *A language and environment for statistical computing*. The R Foundation for Statistical Computing, Vienna, Austria. Accessed at: https://cran.r-project.org/

**Roesler, I., & S. Imberti.** 2015. Abundance and habitat use of Nearctic shorebirds in the highland lakes of western Santa Cruz province, Argentinean Patagonia. *Waterbirds 38*: 86-91.

**Rogers, D.I., P.F. Battley, T. Piersma, J.A. Van Gils & K.G. Rogers.** 2006. High-tide habitat choice: insights from modelling roost selection by shorebirds around a tropical bay. *Animal Behaviour 72*: 563-575.

**Scherer, A.L. & M.V. Petry.** 2012. Seasonal Variation In Shorebird Abundance In The State of Rio Grande Do Sul, Southern Brazil. *The Wilson Journal of Ornithology* 124: 40-50.

**Toldo, Jr, E.E., J.L. Nicolodi, L.E.S.B. Almeida, I.C.S. Corrêa & L.S. Esteves**. 2006. Coastal dunes and shoreface width as a function of longshore transport. *Journal of Coastal Research*, 390-394.

**Verdum, R., L.L.Bier, L.D.F. Santos Vieira & A.A. Formiga**. 2020. Percepção da paisagem e impactos ambientais na geração de energia alternativa em Parques Eólicos no Rio Grande do Sul. *Revista GeoUECE* 9: 7-23.

# ANNEX 1 - SUPPLEMENTARY MATERIAL

UNISINOS

UNIVERSIDADE DO VALE DO RIO DOS SINOS
Programa de Pós-Graduação em Biologia:
Diversidade e Manejo de Vida Silvestre
Laboratorio de Genética e Biologia Molecular

São Leopoldo- RS, 19 de novembro de 2019.

**Sexagem Molecular**

- **Solicitante:** Laboratório de Ornitologia/Unisinos
- **Identificador do indivíduo:** Espécimes de *Calidis canutus*
- **Data emissão do laudo:** 14/11/2019

**Análise** (PCR com *primers* P2 e P8)

**DNA avaliado:**

Este gênero apresenta diferenças de tamanho entre as sequências CHD-1Z/CHD-1W de machos e fêmeas. Sendo assim, espera-se que:

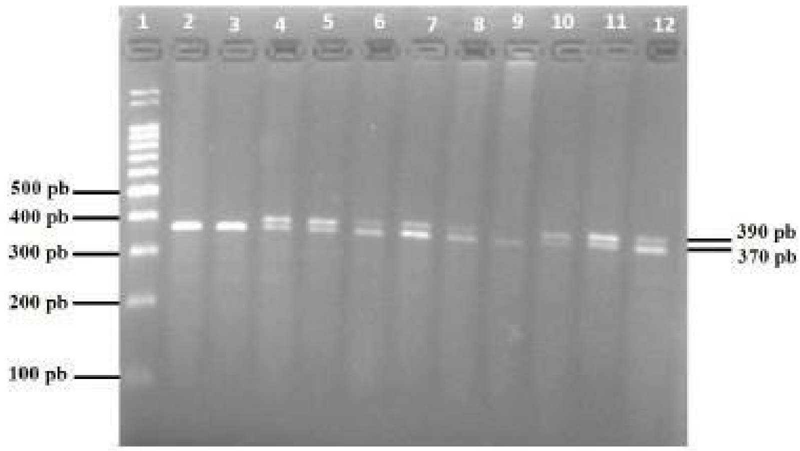
Machos apresentem um único fragmento de 370 pares de bases (pb) correspondente ao gene CHD-Z.

Fêmeas apresentem um fragmento (banda) de 370 pb correspondente ao gene CHD-Z e um segundo fragmento (banda) de 390 pb correspondente ao gene CHD-W.

**Processos:** extração do DNA, amplificação (PCR) do segmento alvo e eletroforese em gel de agarose (duas etapas: extração de DNA e amplificação pel PCR).

**Resultados:** As Figuras mostram o resultado da eletroforese em gel de agarose do produto da PCR dos fragmentos amplificados para os genes CHD-1Z/CHD-1W. **M** (machos); **F** (Fêmeas)

| Lane | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amostra | Ladder | E7Y | E7M | E7X | E1Cb | E1E | E1H | E1Ub | E7A | E1Ca | E9Y | E1Ua |
| Sexo | / | M | M | F | F | F | F | F | M | F | F | F |



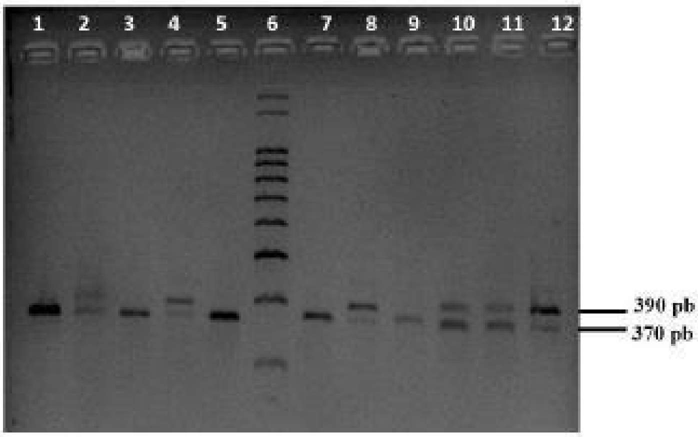

Amostra 1 marcador de tamanho molecular (Ladder 100pb).

Av. Unisinos, 950 Caixa Postal 275 CEP 93022-000 São Leopoldo Rio Grande do Sul Brasil
Fone: (51) 3590-8477 Fax: (51) 3590-8479 - ppgbiologia@unisinos.br - http://www.unisinos.br/mestrado-e-doutorado/biologia

UNISINOS

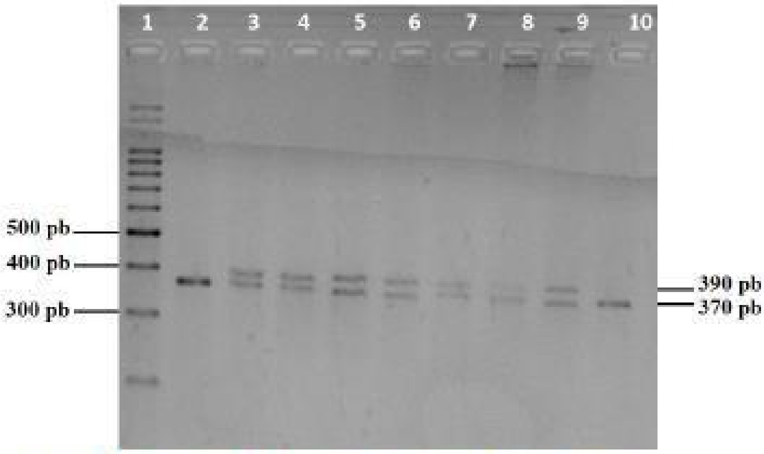
UNIVERSIDADE DO VALE DO RIO DOS SINOS
Programa de Pós-Graduação em Biologia:
Diversidade e Manejo de Vida Silvestre
Laboratorio de Genética e Biologia Molecular

| Lane | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amostra | LCCB1 | LCC41 | E1Y | E2L | E1N | Ladder | E2A | E2J | E2N | E1F | E2K | E2C |
| Sexo | M | F | M | F | M | / | M | F | M | F | F | F |



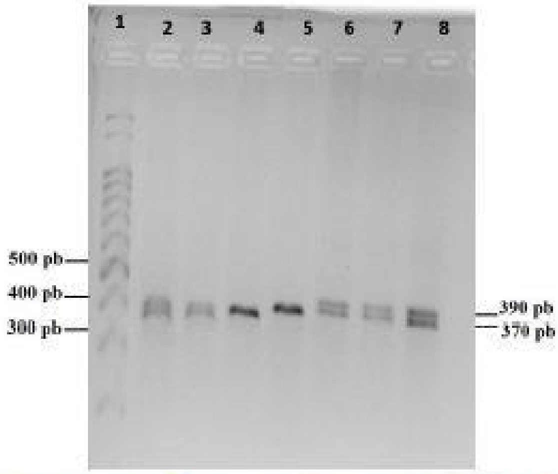

Amostra 6 marcador de tamanho molecular (Ladder 100pb).

| Lane | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Amostra | Ladder | E1J | N=J | E7N | E1T | E7V | E7P | E1K | E8A | E1L |
| Sexo | - | M | F | F | F | F | F | F | F | M |



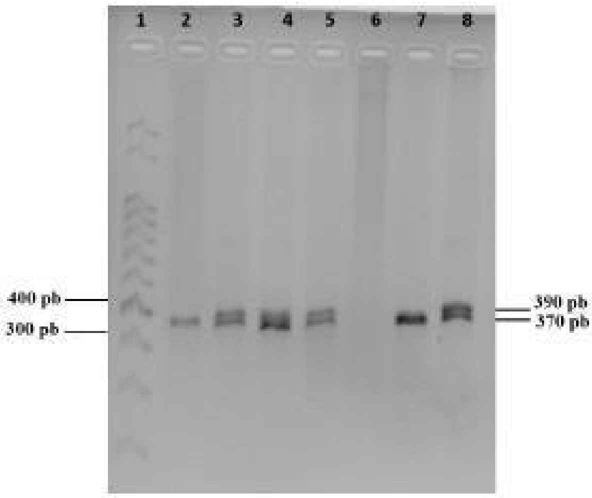

Amostra 1 marcador de tamanho molecular (Ladder 100pb).

| Lane | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Amostra | Ladder | ILP176 | ILP177 | ILP178 | ILP179 | ILP180 | ILP181 | ILP182 |
| Sexo | / | F | F | M | M | F | F | F |

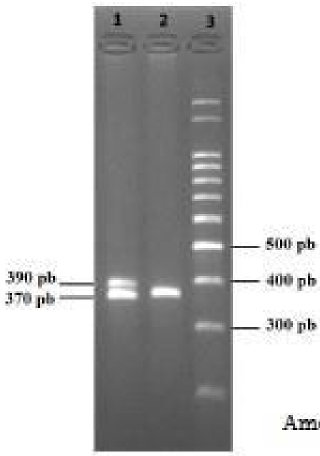



Amostra 1 marcador de tamanho molecular (Ladder 100pb).

| Lane | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Amostra | Ladder | ILP169 | ILP170 | ILP171a | ILP171b | Sem Amostra | ILP174 | ILP175 |
| Sexo | / | M | F | F | F | | M | F |

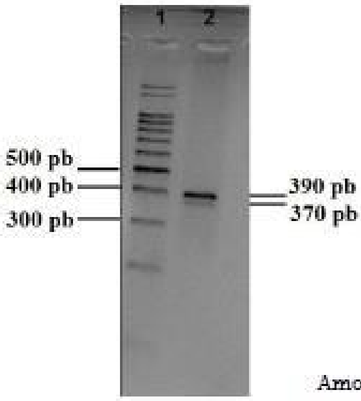



Amostra 1 marcador de tamanho molecular (Ladder 100pb).




| Lane | 1 | 2 | 3 |
|---|---|---|---|
| Amostra | E2Y | E2N | Ladder |
| Sexo | F | M | / |

Amostra 3 marcador de tamanho molecular (Ladder 100pb).




| Lane | 1 | 2 |
|---|---|---|
| Amostra | Ladder | E1A |
| Sexo | - | F |

Amostra 1 marcador de tamanho molecular (Ladder 100pb).

Prof. Dr. Victor Hugo Valiati
Laboratório de Genética e Biologia Molecular E-mail: valiati@unisinos.br

Av. Unisinos, 950 Caixa Postal 275 CEP 93022-000 São Leopoldo Rio Grande do Sul Brasil
Fone: (51) 3590-8477 Fax: (51) 3590-8479 - ppgbiologia@unisinos.br - http://www.unisinos.br/mestrado-e-doutorado/biologia

UNISINOS

UNIVERSIDADE DO VALE DO RIO DOS SINOS
Programa de Pós-Graduação em Biologia:
Diversidade e Manejo de Vida Silvestre
Laboratorio de Genética e Biologia Molecular

São Leopoldo- RS, 26 de setembro de 2020.

# Sexagem Molecular

- **Solicitante:** Laboratório de Ornitologia/Unisinos
- **Identificador do indivíduo:** Espécimes de *Calidis canutus*
- **Data emissão do laudo:** 26/09/2020

**Análise** (PCR com *primers* P2 e P8)

**DNA avaliado**:

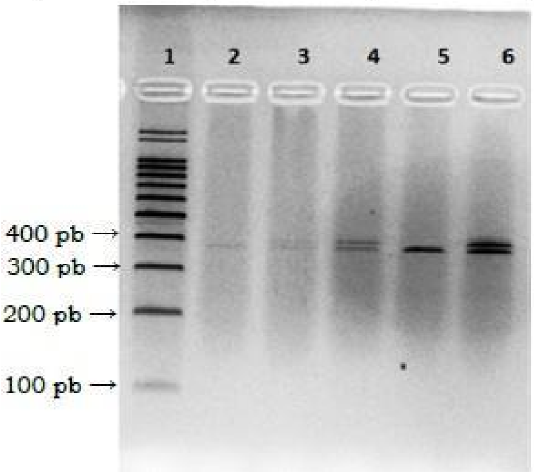
Este gênero apresenta diferenças de tamanho entre as sequências CHD-1Z/CHD-1W de machos e fêmeas. Sendo assim, espera-se que:

Machos apresentem um único fragmento de 370 pares de bases (pb) correspondente ao gene CHD-Z. Fêmeas apresentem um fragmento (banda) de 370 pb correspondente ao gene CHD-Z e um segundo fragmento (banda) de 390 pb correspondente ao gene CHD-W.

**Processos:** extração do DNA, amplificação (PCR) do segmento alvo e eletroforese em gel de agarose (duas etapas: extração de DNA e amplificação pel PCR).

**Resultados:** As Figuras mostram o resultado da eletroforese em gel de agarose do produto da PCR dos fragmentos amplificados para os genes CHD-1Z/CHD-1W. **M** (machos); **F** (Fêmeas)

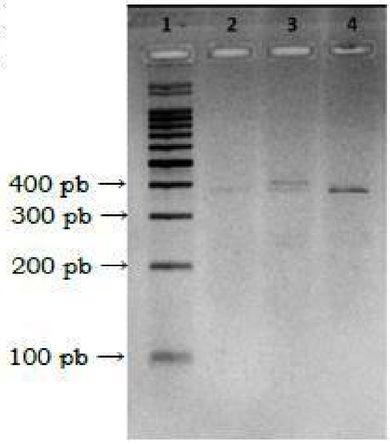
| Lane | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Amostra | Ladder | E1M | E1P | E1X | E2H | V53 |
| Sexo | 100 pb | M | F | F | M | F |



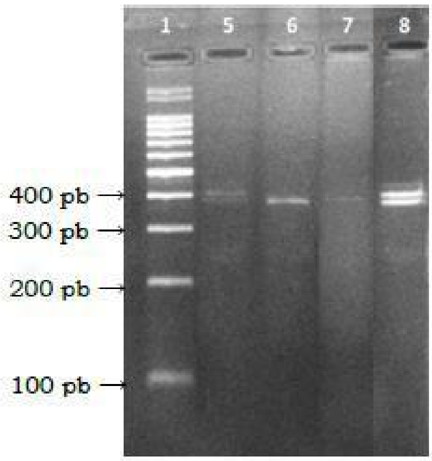
UNISINOS

UNIVERSIDADE DO VALE DO RIO DOS SINOS
Programa de Pós-Graduação em Biologia:
Diversidade e Manejo de Vida Silvestre
Laboratorio de Genética e Biologia Molecular

| Lane | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Amostra | Ladder | E3A | E2M | E2T |
| Sexo | 100 pb | M | F | M |

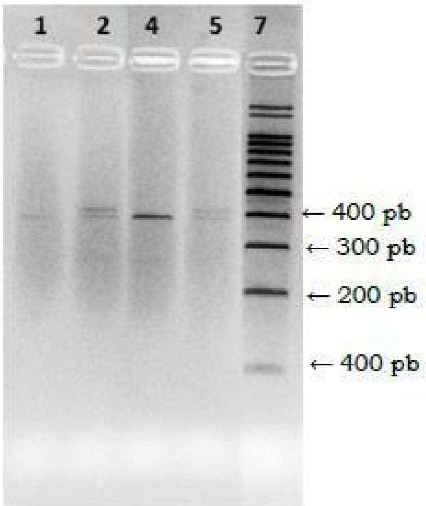



| Lane | 1 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|
| Amostra | Ladder | E2U | E1M | E1P | V53 |
| Sexo | 100 pb | F | M | M | F |




Av. Unisinos, 950 Caixa Postal 275 CEP 93022-000 São Leopoldo Rio Grande do Sul Brasil
Fone: (51) 3590-8477 Fax: (51) 3590-8479 - ppgbiologia@unisinos.br - http://www.unisinos.br/mestrado-e-doutorado/biologia

| Lane | 1 | 2 | 4 | 5 | 7 |
|---|---|---|---|---|---|
| Amostra | E3A | E2M | E2T | E2U | Ladder |
| Sexo | M | F | M | F | 100 pb |




Prof. Dr. Victor Hugo Valiati
Laboratório de Genética e Biologia Molecular E-mail: valiati@unisinos.br