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COMPORTAMENTO DE FORRAGEIO E DIETA DE *Calidris pusilla* DURANTE DIFERENTES DEMANDAS FISIOLÓGICAS DO CICLO MIGRATÓRIO

João Pessoa-PB,
Agosto 2021

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Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas (Zoologia) da Universidade Federal da Paraíba, como requisito para a obtenção do título de Doutor em Ciências Biológicas.

Orientador: Prof. Dr. Helder Farias Pereira de Araujo

Coorientador: Prof. Dr. Gilberto Gonçalves Rodrigues

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Ata da 145ª Apresentação e Banca de Defesa de Doutorado de Leomyr Sângelo Alves da Silva

Ao(s) Trinta e um dias do mês de agosto de dois mil e vinte e um, às 14:00 horas, no(a) Ambiente Virtual, da Universidade Federal da Paraíba, reuniram-se, em caráter de solenidade pública, membros da banca examinadora para avaliar a tese de doutorado de **Leomyr Sângelo Alves da Silva**, candidato(a) ao grau de Doutor(a) em Ciências Biológicas. A banca examinadora foi composta pelos seguintes membros: **Dr. Helder Farias Pereira de Araujo (Orientador - UFPB); Dr. Vitor de Oliveira Lunardi (UFERSA/RN); Dr. Ricardo Romão Guerra (UFPB/PB); Dr. Bráulio Almeida Santos (UFPB/PB); Dr.Patricio Adriano da Rocha (UFPB/PB).** Compareceram à solenidade, além do(a) candidato(a) e membros da banca examinadora, alunos e professores do PPGCB. Dando início à sessão, a coordenação fez a abertura dos trabalhos, apresentando o(a) discente e os membros da banca. Foi passada a palavra ao(à) orientador(a), para que assumisse a posição de presidente da sessão. A partir de então, o(a) presidente, após declarar o objeto da solenidade, concedeu a palavra a **Leomyr Sângelo Alves da Silva**, para que dissertasse, oral e sucintamente, a respeito de seu trabalho intitulado **"Comportamento de Forrageio e Dieta de *Calidris pusilla* Durante Diferentes Demandas Fisiológicas do Ciclo Migratório".** Passando então a discorrer sobre o aludido tema, dentro do prazo legal, o(a) candidato(a) foi a seguir arguido(a) pelos examinadores na forma regimental. Em seguida, passou a Comissão, em caráter secreto, a proceder à avaliação e julgamento do trabalho, concluindo por atribuir-lhe o conceito Aprovado. Perante o resultado proclamado, os documentos da banca foram preparados para trâmites seguintes. Encerrados os trabalhos, nada mais havendo a tratar, eu, orientador(a), como presidente, lavrei a presente ata que, lida e aprovada, assino juntamente com os demais membros da banca examinadora.

João Pessoa, 31/08/21.

Helder F. P. de Araujo
Orientador(a)
Dr. Helder Farias Pereira de Araujo (Orientador - UFPB);

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Examinador(a)
Dr.Patricio Adriano da Rocha (UFPB/PB)

Leomyr Sângelo Alves da Silva
(discente ciente do resultado)

(Em modo de webconferência, as assinaturas digitalizadas são certificadas pelo presidente da banca)

Aos meus queridos pais, Luiz Cardoso e Lucimar Teixeira. A contribuição de vocês para essa fase da minha vida é incalculável. Nessa jornada, eu fiquei com a parte mais fácil, que foi correr atrás dos meus objetivos através dos estudos.

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O homem vangloria-se de ter imitado o voo das aves com uma complicação técnica que elas dispensam.

Carlos Drummond de Andrade.

RESUMO

Todos os anos milhares de aves neárticas realizam grandes migrações entre seus sítios de reprodução, ao norte do Trópico de Câncer, até áreas de invernada, em regiões tropicais e subtropicais. *Calidris pusilla*, uma dessas aves, frequenta principalmente a América do Sul durante sua invernada, utilizando na costa Nordeste do Brasil, regiões marítimas e estuarinas. Nesses locais elas precisam de energia necessária para atender a alta demanda fisiológica do seu ciclo migratório, como troca de plumagem e aumento de massa. Dessa forma, *Calidris pusilla* apresenta flexibilidade nos mecanismos de forrageios para se preparar contra mudanças de habitats, que podem causar imprevisibilidade de presas. Nesse estudo, testamos a hipótese que: as fases que compreendem o ciclo migratório de *Calidris pusilla* na América do Sul, apresentam variações fisiológicas em relação a sua demanda energética, que, consequentemente, influenciam as estratégias de forrageio. Esperamos que as estratégias de forrageio e dieta estejam associadas à demanda energética da ave (troca de plumagem, muda de penas de vôo e contorno), bem como com à disponibilidade de presas no ambiente. O estudo foi conduzido na Ilha da Restinga-PB, ilha fluvial situada no estuário Rio Paraíba, Nordeste do Brasil. A Ilha apresenta uma área de aproximadamente 580 ha, composta principalmente por vegetação de restinga, lagoas e áreas de mangue, a qual os indivíduos de *Calidris pusilla* utilizam para forragear e trocar as suas plumagens. Os resultados dessa pesquisa estão de acordo com a hipótese inicialmente proposta. Encontramos que as fases que compreendem o ciclo migratório de *Calidris pusilla* apresentam variações fisiológicas em relação a sua demanda energética, além disso, *Calidris pusilla* variou o investimento de forrageio para atender as suas demandas fisiológicas, assim como para se prevenir contra imprevisibilidade de presas.

Palavras-Chave: Charadriiformes, Comportamento de forrageio, Índices fisiológicos.

ABSTRACT

Every year thousands of nearctic birds make great migrations between their breeding sites, north of the Tropic of Cancer, to wintering areas in tropical and subtropical regions. *Calidris pusilla*, one of these birds, frequents mainly South America during its wintering period, using maritime and estuarine regions on the northeast coast of Brazil. In these places, they need the energy necessary to meet the high physiological demands of their migratory cycle, such as changing their plumage and increasing their mass. Thus, *Calidris pusilla* presents flexibility in foraging mechanisms to prepare against habitat changes, which can cause prey unpredictability. In this study, we tested the hypothesis that: the phases that comprise the migration cycle of *Calidris pusilla* in South America, present physiological variations in relation to its energy demand, which, consequently, influence foraging strategies. We expect that foraging and diet strategies are associated with the bird's energy demand (change of plumage, flight feather changes and contour), as well as with the availability of prey in the environment. The study was conducted on Ilha da Restinga-PB, a fluvial island located in the Rio Paraíba estuary, Northeastern Brazil. The island has an area of approximately 580 ha, mainly composed of restinga vegetation, lagoons and mangrove areas, which individuals from *Calidris pusilla* use to forage and exchange their plumage. The results of this research are in accordance with the initially proposed hypothesis. We found that the phases that comprise the migratory cycle of *Calidris pusilla* present physiological variations in relation to its energy demand, moreover, *Calidris pusilla* varied the foraging investment to meet its physiological demands, as well as to prevent against prey unpredictability.

Keywords: Charadriiformes, Foraging behavior, Physiological indices.

SUMÁRIO

INTRODUÇÃO GERAL

Calidris pusilla (Linnaeus, 1758) (Aves: Scolopacidae) é uma das espécies de aves neárticas mais amplamente distribuída na América do Norte, que reproduz no ártico Canadense e Alasca, frequentando principalmente a América do Sul durante o período de invernada (Gratto-Trevor et al. 2012). Na costa nordeste do Brasil é uma das aves mais abundantes em regiões estuarinas e de fácil captura (Rodrigues 2000, Cardoso & Zeppelini 2011). No entanto, mesmo sendo uma espécie abundante, *Calidris pusilla* tem requerido maiores atenções recentemente, por causa do registro de grande declínio populacional nas últimas décadas, tanto nas áreas de reprodução, quanto nas de invernada (Morrison et al. 2006, 2012).

Sabe-se que durante o ciclo migratório, *Calidris pusilla* necessita de alimentação adequada para suprir a alta demanda energética e reconstruir as suas reservas fisiológicas para as longas migrações (Schaub et al. 2008). A sua dieta pode ser variada, composta principalmente de macroinvertebrados como: poliquetas, bivalves, gastrópodes e crustáceos (Hicklin & Smith 1979, Skagen & Oman 1996, Maillet & Weber 2006). Para se adaptar a variedade de presas, frente as grandes flutuações abióticas de áreas estuarinas, essa ave apresenta alta plasticidade de forrageio, com mecanismos bem conhecidos no gênero *Calidris*, como: bicar – penetração rasa do bico (¼ do bico no sedimento ou menos) e sondar – penetração mais profunda do bico (mais que ¼ do comprimento do bico no sedimento) (Baker & Baker 1973).

Na costa nordeste do Brasil, *Calidris pusilla* chega após longos vôos migratórios, troca sua plumagem reprodutiva para uma plumagem de invernada e, cerca de dois meses antes do retorno para o hemisfério norte, troca novamente a plumagem para a plumagem reprodutiva, mudam as penas de voo e acumula cerca de 40% a mais de sua massa corporal. No entanto, essa ave precisa finalizar o processo de mudas de penas para atingir a massa necessária para o voo migratório de retorno, devido à demanda energética exigida nessa troca de penas (Rodrigues et al. 2009, Rodrigues et al. 2016).

Nesse sentido, espera-se que as estratégias de forrageio e dieta estejam associadas à demanda energética da ave (troca de plumagem, muda de penas de vôo e contorno), bem como com à disponibilidade de presas no ambiente. Além dos resultados dessa pesquisa poderem elucidar questões de interesses adaptativos relacionados à biologia da migração, podem fornecer informações importantes da biologia de *Calidris pusilla* que auxiliem no entendimento dos motivos desse declínio populacional relatado nas últimas décadas.

A utilização de parâmetros hematológicos serão empregados como ferramenta para medir o estresse fisiológico de *C. pusilla* durante a invernada na América do Sul. Em pesquisas ecológicas, o uso desses parâmetros está associado às variações dos perfis leucocitários dos vertebrados, representados, em grande escala, por neotrófilos/heterofilos e linfócitos (Davis et al. 2008, Minias et al. 2017). É utilizado à relação desses dois leucócitos para medir os níveis de estresse de acordo com as demandas fisiológicas influenciadas por algum período ou flutuações do ambiente (Davis et al. 2008).

A estruturação da tese está direcionada em capítulos, testando a hipótese geral: as fases que compreendem o ciclo migratório de *Calidris pusilla* na América do Sul (chegada do Hemisfério Norte, estabilização e migração de saída), apresentam variações de estresse fisiológico em relação a sua demanda energética (troca de plumagem, muda de penas de vôo, acúmulo de massa na preparação para migração) que, consequentemente, influenciam as estratégias de forrageio.

Dessa forma, o primeiro capítulo tem como título: “ Perfis heterofilos/linfócitos são associados com o aumento de massa e muda de maçarico-rasteirinho (*Calidris pusilla*) em sítios de invernada no NE da América do Sul ”.

O segundo capítulo, apresenta o título: “Comportamento oportunista e variação no investimento de forrageio de maçarico-rasteirinho (*Calidris pusilla*) durante inverno na América do Sul”.

O terceiro capítulo, traz o seguinte título: “Influência da variação de recursos alimentares no investimento de forrageio e indicadores de estresse de maçarico-rasteirinho (*Calidris pusilla*) durante o inverno”.

REFERÊNCIAL TEÓRICO

- *Mecanismos fisiológicos de estresse (Taxas H/L)*

As ferramentas que promovem a compreensão de respostas fisiológicas ao meio ambiente, estão se tornando cada vez mais frequente para ecólogos que buscam entender padrões da biologia de animais selvagens (Davis et al. 2008). O desenvolvimento desses aspectos levou a formação de uma disciplina recente, denominada "fisiologia da conservação" (Stevenson et al. 2005, Wikelski & Cooke 2006).

Nas últimas décadas, métodos complementares de estresse fisiológico, como os parâmetros hematológicos, que consistem na contagem relativa de leucócitos preparadas a partir de esfregaços de sangue, tem se tornado cada vez mais popular devido as vantagens em relação aos hormônios glicocorticoides, especialmente no estudo de aves (Hawkey & Dennett 1989, Jain 1993, Thrall 2004, Davis et al. 2008). Em geral, grande parte dos táxons tem cinco tipos de leucócitos: linfócitos, neutrófilos, eosinófilos, basófilos e monócitos (Hawkey & Dennett 1989, Jain 1993). No entanto, em pássaros e répteis o neutrófilo é substituído pelo heterofilo, a qual apresenta a mesma função imunológica (Jain 1993). Dessa forma, aproximadamente 80% de leucócitos nos vertebrados é constituído por neutrófilo/heterofilo e linfócitos (Davis et al 2008).

Os linfócitos apresentam uma variedade de funções imunológicas: 1) células assassinas, que participam da defesa imune inata contra células tumorais ou vírus, 2) células T, que apresentam um papel importante na defesa adaptativa e 3) células B, que são responsáveis pela produção de imunoglobulinas que reconhecem patógenos (Genovese 2013). Já os neutrófilos/heterofilos, formam a primeira linha de defesa celular inata contra patógenos, além disso participam ativamente contra inflamações utilizando um repertório vasto de mecanismo como: fagocitose, degranulação celular ou explosão oxidativa (Davis et al. 2008, Johnstone et al. 2012, Genovese 2013).

Assim, com as várias respostas de neutrófilos/heterofilos e linfócitos a estressores externos, foi proposto que a sua relação poderia ser útil na detecção do estresse fisiológico em vertebrados (Stevenson et al. 2005, Wikelski & Cooke 2006, Davis et al. 2008, Johnstone et al. 2012). Em ambientes estressantes, com o estimulo de glicocorticoides, ocorre uma diminuição dos linfócitos no sangue, transmigrando para o baço, pele e nódulos linfáticos, onde serão mais úteis em estado de lesão, ao passo que é visto um aumento de neutrófilos/heterofilos no sangue circulante, essas mudanças são para garantir que os tipos celulares sejam encaminhados para onde sejam necessárias durante a resposta ao estresse (Dhabhar et al. 1994, 1996). A consequência da relação desses dois leucócitos, indica para as maiores proporções uma resposta mais forte ao estresse (Davis et al. 2008, Johnstone et al. 2012).

De acordo com Johnstone et al. 2012, que revisaram a interpretação desse índice, o mecanismo responsável por essa mudança está possivelmente relacionado a variações na fisiologia do animal de um estado de prontidão para lidar com uma doença transmissível a um estado de preparação para infecção por lesão. Assim, o animal se aclimata para um ambiente com maior risco de lesão: ex. a ação predadora ou, para uma situação em que o animal é incapaz de evitar lesões: ex. más condições do ambiente.

Em aves a variação dos índices de estresse (taxas H/L) foi relatada para refletir um grande espectro de estressores. Foram relatadas altas taxas H/L para infestação de parasitas (Davis et al. 2004, Lobato 2005), poluição de hábitats (Eeva et al. 2005), fragmentação de habitats (Hinam & Clair 2008), Urbanização (Fokidis et al. 2008) e aumento do esforço de reprodução (Ilmonen et al. 2003). Além desses, as taxas H/L se correlacionaram negativamente a taxa de crescimento dos filhotes (Moreno 2002), estado nutricional (Suorsa et al. 2004), status de dominância dentro da ninhada (Laaksonen et al. 2004) e condição adulta (Hanssen et al. 2003). Em aves da ordem Charadriiformes, foi visto recentemente em *Calidris pusilla* uma relação positiva entre taxas H/L e as mudas e, uma relação negativa entre taxas H/L e aumento de massa, ambos durante o período de invernada e preparação para migração respectivamente (Silva et al. 2021). Assim, estudos que busquem entender a relação entre mecanismos fisiológicos e as variáveis biológicas dessas aves, podem ajudar a compreender lacunas de conhecimento na sua história de vida.

- *Biologia de aves limícolas*

Todos os anos milhares de aves limícolas neárticas realizam migrações sazonais a partir de seus locais de reprodução, ao norte do trópico de câncer, para passarem o inverno boreal nas regiões tropicais e subtropicais, onde utilizam principalmente as costas marítimas dos continentes, entre o período de setembro à maio (Harrington e Morrison 1979, Newton 2007).

Nessas áreas, as aves se alimentam e estocam energia para vários processos fisiológicos do seu ciclo migratório, como troca de plumagem, mudas de penas de vôo e acúmulo de massa suficiente para permitir longos vôos de volta para os sítios reprodutivos (Placyk & Harrington 2004, Rodrigues et al. 2009). Desta forma, a massa corporal torna-se um parâmetro essencial para ajudar a entender diversos aspectos relativos ao ciclo migratório (Zwarts et al. 1990).

Alguns estudos têm abordado a alta capacidade que as aves limícolas apresentam em aumentar consideravelmente sua massa corporal em poucas semanas ou dias, refletida principalmente no acúmulo de gordura e aumento de órgãos internos e grupos musculares, no período que antecede a sua migração (Piersma et al. 1999, Battley & Piersma 2005). Consequentemente, tais modificações correspondem, proporcionalmente, as mais rápidas alterações corporais dentre qualquer outro grupo de vertebrado (Alerstam & Lindström 1990).

Ao longo do ano, com as diversas necessidades entre as fases da migração, o sistema digestivo das aves limícolas precisa ser morfologicamente flexível para lidar com a disponibilidade de recursos alimentares e as exigências nutricionais necessárias (Battley & Piersma 1997, 2005). Desta forma, o funcionamento interno das aves estará em constantes alterações em relação as várias demandas energéticas (Battley & Piersma 2005), possivelmente associado à variação de estratégias comportamentais de forrageio e dieta frente as variações sazonais e espaciais nos ambientes que usam (Martins et al. 2013).

Nas áreas estuarinas, onde se observam vários habitats naturais com recursos alimentares importantes para as aves limícolas neárticas (Warnock 2001, Newton 2007), a disponibilidade de macroinvertebrados tende a variar devido as condições abióticas, com os picos de abundância e biomassa distribuindo-se de forma irregular durante todo o ano (Kober & Bairlein 2006).

Para se adaptarem as grandes flutuações da abundância e acessibilidade de macroinvertebrados, essas aves apresentam alta plasticidade comportamental e exploram recursos alimentares variados durante o seu ciclo migratório (Beninger et al. 2011). A seleção de macroinvertebrados por aves limícolas pode ocorrer a partir dos teores de nutrientes, independente da baixa densidade de presas, como acontece com algumas espécies de crustáceos, que são ricos em ácidos polissaturados (Maillet & Weber 2006). Em alguns casos, a dieta pode ser oportunista, onde as aves se alimentam de acordo com a disponibilidade de macroinvertebrados (Davis & Smith 2001).

A alta flexibilidade de forrageio permite com que essas aves se protejam contra declínios inesperados de alimentos (Baillie & Jones 2004). Dessa forma, entender como esses processos estão relacionados a demanda energética, que ocorre em aves limícolas durante o ciclo migratório na América do Sul, pode ajudar a elucidar padrões utilizados por essas aves que contribuem para o seu sucesso migratório.

REFERÊNCIAS

Alerstam, T. & Lindström, A. Optimal bird migration: the relative importance of time, energy, and safety. In: **Bird Migration: Physiology and Ecophysiology** (ed. Gwinner. Berlin: Springer-Verlag), p. 331–351, 1990.

Baillie, S.M., & I. L. Jones. Response of atlantic Puffins to a decline in capelin abundance at the Gannet Islands, Labrador. **Waterbirds** 27.102-111, 2004.

Baker, M. C., & A. E. M. Baker. 1973. Niche relationships among six species of shorebirds on their wintering and breeding ranges. **Ecological Monographs** 43:193–212.

Battley, P.F. & Piersma, T. Body composition and flight ranges of bar-tailed godwits (*Limosa lapponica baueri*) from New Zealand. **The Auk** 122(3): 922-937, 2005.

Battley, P.F. & Piersma, T. Body composition of lesser knots (*Calidris canutus rogersi*) preparing for take-off on migration from northern New Zealand. **Notornis** 44:137–150, 1997.

Beninger, P.G., Elner R.W., Morancais M., Decottignies, P. Downward trophic shift during breeding migration in the shorebird *Calidris mauri* (western sandpiper). **Marine Ecology Progress Series** 428: 259–269, 2011.

Cardoso, T.A.L. & Zeppelini, D. Migratory shorebirds during boreal summer and southward migration on the coast of Paraíba, Brazil. **Waterbirds** 34: 369–375, 2011.

Davis, A. K., Cook, K. C., & Altizer, S. Leukocyte profiles in wild house finches with and without mycoplasmal conjunctivitis, a recently emerged bacterial disease. **EcoHealth**, **1**, 362– 373, 2004.

Davis, C. A., & L. M. Smith. Foraging strategies and niche dynamics of coexisting shorebirds at stopover sites in the southern Great Plains. **The Auk** 118:484–495, 2001.

Davis, A.K., Maney, D.L. & Maerz, J.C. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. **Functional Ecology**, 22, 760−772, 2008.

Dhabhar, F. S., Miller, A. H., McEwen, B.S. & Spencer, R. L. Stressinduced changes in blood leukocyte distribution – role of adrenal steroid hormones. **Journal of Immunology**, 157, 1638 –1644, 1996.

Dhabhar, F. S., Miller, A. H., Stein, M., McEwen, B. S. & Spencer, R.L. Diurnal and acute stress-induced changes in distribution of peripheral blood leukocyte subpopulations. **Brain Behavior and Immunity**, 8, 66 –79, 1994.

Eeva, T., Hasselquist, D., Langefors, Å., Tummeleht, L., Nikinmaa, M., & Ilmonen, P. Pollution related effects on immune function and stress in free-living population of pied flycatcher *Ficedula hypoleuca*. **Journal of Avian Biology**, 36, 405– 412, 2005.

Fokidis, H. B., Greiner, E. C., & Deviche, P. Interspecific variation in avian blood parasites and haematology associated with urbanization in a desert habitat. **Journal of Avian Biology**, 39, 300– 310, 2008.

Genovese, K. J., Swaggerty, C. L., & Kogut, M. H. The avian heterophil. **Developmental and Comparative Immunology**, 41, 334– 340, 2013.

Gratto-Trevor, C., Guy M.R.I., Mizrahi. D., Lank, D.B., Hicklin, P. & Arie L. Migratory Connectivity of Semipalmated Sandpiper: Winter Distribution and Migration Routes of Breeding Populations Spaans Source: **Waterbirds**, 35(1):83-95. 2012.

Hanssen, S. A., Folstad, I., & Erikstad, K. E. Reduced immunocompetence and cost of reproduction in common eiders. **Oecologia**, 136, 457– 464, 2003.

Harrington, B.A. & Morrison, R.I.G. Semipalmated Sandpiper (*Calidris pusilla*). **Wader Study Group Bulletin** 33: 37-38, 1979.

Hawkey, C.M. & T. B. Dennett (1989). Color Atlas of Comparative Veterinary Hematology. **Iowa State University Press,** Ames, Iowa.

Hicklin, P.W., & Smith, P. C. The diets of five species of migrant shorebirds in the Bay of Fundy. **Proc. N.S. Inst. Sci.** 29, 483–488, 1979.

Hinam, H. L., & St. Clair, C. C. High levels of habitat loss and fragmentation limit reproductive success by reducing home range size and provisioning rates of Northern saw-whet owls. **Biological Conservation**, 141, 524– 535, 2008.

Ilmonen, P., Hassequist, D., Langefors, Å., & Wiehn, J. Stress, immunocompetence and leukocyte profiles of pied flycatchers in relation to brood size manipulation. **Oecologia**, 136, 148– 154, 2003.

Jain, N. C. (1993) Essentials of Veterinary Hematology. **Blackwell Publishing**, Philadelphia, PA.

Johnstone, C. P., Reina, R. D., & Lill, A. J. Interpreting indices of physiological stress in free-living vertebrates. **Journal of Comparative Physiology B**, 182, 861– 879, 2012.

Kober. K., & F. Bairlein. Shorebirds of the Bragantinian Peninsula in prey availability and shorebird consumption at a tropical site in northern Brazil. **Ornitologia Neotropical** 17: 531–548, 2006.

Laaksonen, T., Fargallo, J. A., Korpimäki, E., Lyytienen, S., Valkama, J., & Pöyri, V. Year- and sex-dependent effects of experimental brood sex ratio manipulation on fledging condition of Eurasian kestrels. **Journal of Animal Ecology**, 73, 342– 352, 2004.

Lobato, E., Moreno, J., Merino, S., Sanz, J. J., & Arriero, E. Haematological variables are good predictors of recruitment in nestling pied flycatchers (*Ficedula hypoleuca*). **Ecoscience**, **12**, 27– 34, 2005.

Martins, R.C., Catry, T., Santos, C.D., Palmeirim, J.M., Granadeiro, J.P. Seasonal Variations in the Diet and Foraging Behaviour of Dunlins *Calidris alpina* in a South European Estuary: Improved Feeding Conditions for Northward Migrants. **Plos One**

8(12): e 81174, 2013.

Maillet, D., & J. M. Weber. Performance-enhancing role of dietary fatty acids in a long-distance migrant shorebird: the Semipalmated Sandpiper. **Journal of Experimental Biology,** 209, 2686 – 2695, 2006.

Minias, P., Włodarczyk, R., & Meissner, W. Leukocyte profiles are associated with longevity and survival, but not migratory effort: A comparative analysis of shorebirds. **Functional Ecology**, 32, 369-378, 2017.

Moreno, J., Merino, S., Martínez, J., Sanz, J., & Arriero, E. Heterophil/lymphocyte ratios and heat-shock protein levels are related to growth in nestlings birds. **Ecoscience**, 9, 434– 439, 2002.

Morrison, R. I. G.; Mizrahi, D. S., Ross, R. K., Ottema, O. H., Pracontal N. & Narine, A. Dramatic declines of Semipalmated Sandpiper on their major wintering areas in the Guianas, northern South America. **Waterbirds** 35: 120-134, 2012.

Morrison, R. I. G., Mc Caffery, B. J., Gill, R. E., Skagen, S. K., Jones, S. L., Page, G.W., Gratto- Trevor, C. L., Andres, B.A. Population estimates of North American shorebirds. **Wader Study Group Bull**, 2006.

Newton, I. The migration ecology of birds. Londres: **Academic Press**. 2007.

Placyk, J.J.R & Harrington, B.A. 2004. Prey abundance and habitat use by migratory shorebirds at coastal stopover sites in Connecticut**. Journal of Field Ornithology** 75(3): 223-231.

Piersma, T.; Gudmundsson, G.A.; & Lilliendahl, K. Rapid changes in the size of different functional organ and muscle groups during refuelling in a long-distance migrating shorebird. **Physiological and Biochemical Zoology** 72, 405-415, 1999.

Rodrigues, A.A.F. Seazonal abundance of neartic shorebirds in the gulf of Maranhão, Brazil. **Journal of Field Ornithology** 71,4, 665-675, 2000.

Rodrigues, R.C.; Azevedo J.; Larrazabal M.M. & Araujo, H.F.P. Temporal variations of body mass and plumage in *Arenaria interpres* (Aves, Scolopacidae) along the Brazilian coast. **Zoologia** 26: 386-390, 2009.

Rodrigues, R. C., de Araujo, H. F. P., Guerra, R. R., Durigon, E. L., Mizrahi, D. S., and de Azevedo Junior, S. M. Temporal variation in the mass and plumage of four Charadriiformes species on the north-eastern coast of Brazil. **Emu-Austral Ornithology**, 116(4), 461-466, 2016.

Silva L. S. A., Rafael R. M., Rodrigues G. G., de Araujo H. P. Heterophile/lymphocyte profiles are associated with mass increase and moulting in the Semipalmated Sandpiper *Calidris pusilla* at wintering sites in NE South America. **Acta Ornithol.** 56: 127–132, 2021.

Schaub, M., Jenni, L., and Bairlein, F. Fuel stores, fuel accumulation, and the decision to depart from a migration stopover site. **Behavioral Ecology,** 19, 657– 666, 2008.

Skagen, S. K., and Oman, H. D. Dietary flexibility of shorebirds in the western hemisphere. **Can. Field-Nat**. 110, 419-444, 1996.

Stevenson, R. D., Tuberty, S. R., DeFur, P. L. & Wingfield, J.C. Ecophysiology and conservation: the contribution of endocrinology and immunology – introduction to the symposium. **Integrative and Comparative Biology**, v. 45, p. 1– 3, 2005.

Suorsa, P., Helle, H., Koivunen, V., Huhta, E., Nikula, A., & Hakkarainen, H. Effects of forest patch size on physiological stress and immunocompetence in an area-sensitive passerine, the Eurasian treecreeper (*Certhia familiaris*): An experiment. **Proceedings of the Royal Society B**, 271, 435– 440, 2004.

Thrall, M.A. (2004) Hematology of amphibians, Veterinary Hematology and Clinical Chemistry: **Text and Clinical Case Presentations**. Lippincott Williams & Wilkins, Philadelphia, PA.

Warnock, S. E.; Takekawa. J. Y. Habitat preferences of wintering shorebirds in a temporally changing environment: Western Sandpipers in the San Francisco Bay estuary. **The Auk**, v. 112, p. 920-930, 2001.

Wikelski, M. & Cooke, S. J. Conservation physiology. **Trends in Ecology and Evolution**, 21, 38 – 46, 2006.

Zwarts, L., Kersten, M. & Piersma, T. Moult mass and flight range of waders ready to take off long-distance migrations. **Ardea** 78: 339-364, 1990.

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CAPÍTULO 1

Perfis heterofilos/linfócitos são associados com o aumento de massa e muda de maçarico-rasteirinho (*Calidris pusilla*) em sítios de invernada no NE da América do Sul

AGOSTO/2021

Heterophile/lymphocyte profiles are associated with mass increase and moulting in Semipalmated Sandpiper (*Calidris pusilla*) at wintering sites in NE South America

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Heterophile/lymphocyte profiles are associated with mass increase and moulting in Semipalmated Sandpiper (*Calidris pusilla*) at wintering sites in NE South America

Abstract

Leukocyte profiles, such as the heterophile/lymphocyte (H/L) ratio, are influenced by several ecological and ecophysiological factors and are generally considered to be independent and robust indicators of stress levels in birds. We tested the hypotheses that Semipalmated Sandpiper *Calidris pusilla* presents a positive association between physiological stress (H/L ratio) and molt, and that there should be a negative association between physiological stress (H/L ratio) and body condition (mass). During its annual cycle, Semipalmated Sandpiper performs long-distance migrations and often arrives in South America in September/October when it can undergo molting before its migration to the north. In May, while preparing to return to its reproductive sites, Semipalmated Sandpiper increases its body mass. The research was carried out on Restinga Island of the Rio Paraíba estuary in northeast Brazil. Data collection took place during the wintering of Semipalmated Sandpiper in October 2017 and February and May 2018. We found a positive association between the H/L ratio and the presence of molt in October and February, but a negative association between the H/L ratio and body mass in May. Our results support the hypotheses that the presence of molt should be positively associated with the H/L ratio due to the high energy and nutritional costs that molting requires and that the H/L ratio and body mass should be negatively associated due to the favorable nutritional status of Semipalmated Sandpiper during its preparation to migrate to its reproductive areas.

Keywords: Charadriiformes, body condition, stress indices, shorebirds migration.

INTRODUCTION

The heterophile/lymphocyte (H/L) ratio is considered a robust measure of physiological stress in birds and is becoming an important tool in ecological and ecophysiological studies (Wikelski &Cooke 2006, Davis et al. 2008). This physiological parameter changes in response to stress hormones, such as corticosterone, which stimulate lymphocytes to transmigrate from blood to other compartments of the body (e.g., skin, spleen, lymph nodes). Meanwhile, inflows of heterophile are boosted to transmigrate from bone marrow to blood (Müller et al. 2011, Johnstone et al. 2012). In this way, the organism adapts to a stressful environment, which presents a higher risk of injury (Johntone et al. 2012). In this way, the organism adapts to a stressful environment, which presents a higher risk of injury (Johntone et al. 2012). Increases in the H/L ratio are known to reflect physiological stress due to parasite infestations (Davis et al. 2004, Lobato et al. 2005), fragmentation of natural habitats (Fokidis et al. 2008, Hinam & St. Clair 2008), pollution (Eeva et al. 2005), breeding efforts (Ilmonen et al. 2003), and molting (Pap et al. 2010), among other factors (Johnstone et al. 2012).

The energy and nutritional costs of molting are among the most stressful processes of the annual cycle of waders Charadriiformes, which are known for traveling long distances during migration (Lindström et al. 1993). The synthesis of new feathers involves an increase in the metabolism of amino acids due to the deposition of proteins, which constitute up to 25% of the lean body mass of this group (Murphy & Taruscio 1995). However, Włodarczyk et al. (2018) found that leukocyte profiles in a short-distance migrant, the Common Snipe *Gallinago gallinago*, were related to nutritional condition, but not to molting stress. In this case, body condition, including mass, fat load, plasma concentrations of triglycerides, and total protein were negatively related to physiological stress indicators. Since variation in the mechanisms of physiological stress response can occur among Charadriiformes species, these different relationships need to be investigated, especially in long distance migrants.

Semipalmated Sandpiper *Calidris pusilla*, one of the species of Charadriiformes, arrives on the north/northeast coast of South America with low body reserves after migration from its breeding grounds, mostly in Canada and Alaska and does not show much variation in body mass until it prepares for its return to the northern hemisphere (Rodrigues et al. 2016). Semipalmated Sandpiper presents a non-reproductive plumage at the start of the wintering period (September/October) and gradually changes to intermediate plumage in the middle of wintering (Rodrigues et al. 2016). While preparing for its return to the northern hemisphere, usually with a full reproductive plumage, Semipalmated Sandpiper significantly increases its body mass to make the approximately 2,800 km-flight to the areas in the north of South America (Rodrigues 2000, Brown & Jordan 2014). After undertaking these flight efforts, Semipalmated Sandpiper continues its migration toward Canada with temporary stops in Central America and the United States (Brown & Jordan 2014).

In the present study, we aimed to analyze whether the energy and nutritional cost of molting during the wintering period increases the physiological stress of the sandpiper. For this, we tested two hypotheses: 1) Semipalmated Sandpiper physiological stress increases in response to molt, resulting in a positive association between physiological stress (H/L ratio) and the presence of molt and 2) Semipalmated Sandpiper develops a favorable nutritional status (high mass) in order to return to the northern hemisphere, which should decrease physiological stress, resulting in a negative association between H/L ratio and body condition (mass). We hope to fill a knowledge gap in the ecology of Semipalmated Sandpiper migration by demonstrating how physiological processes are related to the energy requirements of the migratory cycle.

METHODS

Study area

The field activity of this work was carried out on Restinga Island, a fluvial island located in the Rio Paraíba estuary in northeast Brazil (Fig. 1). The island has an area of approximately 580 ha and is mainly composed of lagoons, and mangrove areas. Much of the island is protected by a private initiative that develops ecotourism activities. Large groups of Semipalmated Sandpiper rest and forage throughout the wintering period along the island's temporal lakes and sandbanks (Cardoso & Zeppelini 2011).

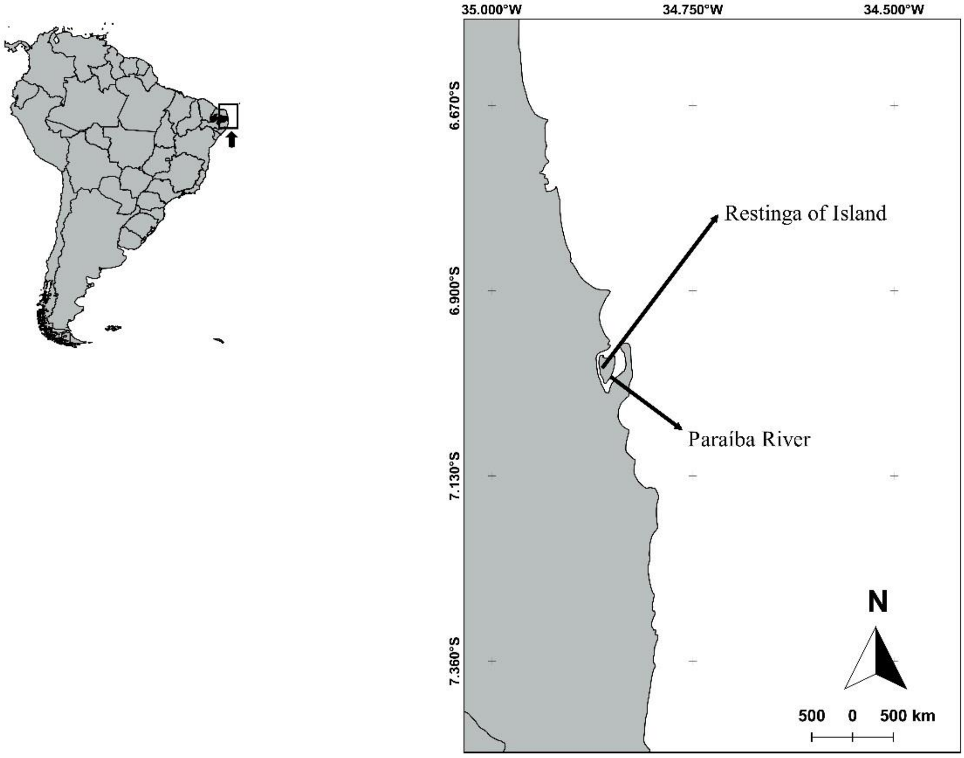


Fig. 1. Map of the geographical location of the study size, Restinga Island, northeast Brazil.

Catching birds

Data was collected on the northeast coast of South America in 2017 and 2018 after the arrival of Semipalmated Sandpiper (October), in the mid-period (February), and during preparations for the migration to the north (May). The birds were captured using mist nets and marked with a metal ring provided by Centro Nacional de Pesquisa e Conservação de Aves (CEMAVE/ICMBio). Data was collected from a total of 85 *C. pusilla* individuals during their wintering in the study area (30 in October, 28 in February, and 27 in May). Semipalmated Sandpiper individuals were captured only once during the data collection period.

As a part of the post-capture procedures, body mass data was taken with dynamometer-type scales and an accuracy of 1g. Incidence data for feather molting was obtained from the contour feathers (head, back and belly) and the flight feathers (wings and tail) (Ginn & Melville 1983). Semipalmated Sandpiper presented non-reproductive plumage in October and February and full reproductive plumage in May, as recorded in the wintering areas on the northeast coast of South America (Rodrigues et al. 2016).

Blood smears

Blood sample collection was conducted first after the capture procedures to decrease the possibility of changes in blood parameters due to stress caused by the capture and handling process (Davis 2005). Blood smears were made from the blood taken from the brachial vein of the Semipalmated Sandpiper individuals within a maximum of 30 minutes after capture. The smears were immediately prepared on slides and stained using the May-Grunwald-Giemsa (MGG) method.

We evaluated 100 fields of view in each blood smears and counted each leukocyte through microscopic observation at 100x magnification using the oil immersion technique. The 100 fields of view were chosen based on the homogeneous distribution of the red blood cells, disregarding the outside areas of the smear due to the edge effect (Owen & Moore 2006). Immunological ratios were calculated from these counts. We used H/L ratios as indicators of physiological stress related to energy demand (Johnstone et al. 2012).

The eosinophil and monocyte counts were used to check whether any interference from pathogens might have influenced or masked the variation of the H/L ratios in the periods evaluated in the present study. The increase in leukocytes in the blood smears was a useful indicator of health alterations in the birds related to parasites (eosinophils) and bacteria (monocytes) (Davis et al. 2008).

Statistical analysis

To test associations of four variables — 1) presence/absence of molt, 2) body mass, 3) eosinophils, and 4) monocytes — with the response variable (H/L ratio), we used the general linear models (GLMs) with significance threshold of p = 0.05 and a normal distribution (family = gaussian). We analyzed the data from each month of data collection: arrival (October), mid-period (February), and preparation for migration (May). All analyses were performed on the R platform, version 3.5.0. (R Core Team 2018) The GLM analysis was performed using the nlme statistical package (linear and nonlinear mixed effects models) (Bates & Pinheiro 2018).

RESULTS

The relation between the H/L ratio and the presence of molt was significantly positive for Semipalmated Sandpiper individuals in October (arrival) and February (mid-period) (Table 1, Fig. 2). However, we found a non-significant negative trend between body mass and H/L ratio during the mid-period, and marginally non-significant negative association between body mass and H/L ratio was apparent during the arrival period (Table 1). Meanwhile, we found a significantly negative association between body mass and H/L ratio in May (preparation for migration) (Table 1, Fig. 3). We did not find any significant associations between the H/L ratio and eosinophils and monocytes in any evaluated period (Table 1).

Table 1. Generalized Linear Models of the H/L rate in relation to molt, mass variation, and variation in eosinophils and monocytes of the Semipalmated Sandpiper during wintering period in South America.

Predictors	**T**	**P**	***B***
October (Arrival)			
Moult (presence/absence)	2.057	0.049	2.823
Mass	-1.771	0.087	-0.637
Eosinophils	-1.760	0.089	-0.527
Monocytes	-0.745	0.463	-0.536
February (Mid-period)			
Moult (presence/absence	2.452	0,021	5.394
Mass	-0.961	0.346	-0.446
Eosinophils	-0.984	0.144	-1.505
Monocytes	-0.984	0.144	-1.505
May (Pre-migration)			
Mass	-0.639	0.001	-0.387
Eosinophils	-0.292	0.773	-0.007
Monocytes	-0.831	0.414	-0.020

Fig. 2. Heterophile/lymphocyte ratios of Semipalmated Sandpiper with and without feather molting in October (Arrival) and February (Mid-period) at wintering sites on the northeast coast of South America. Note different scales of Y axes in left and right panels

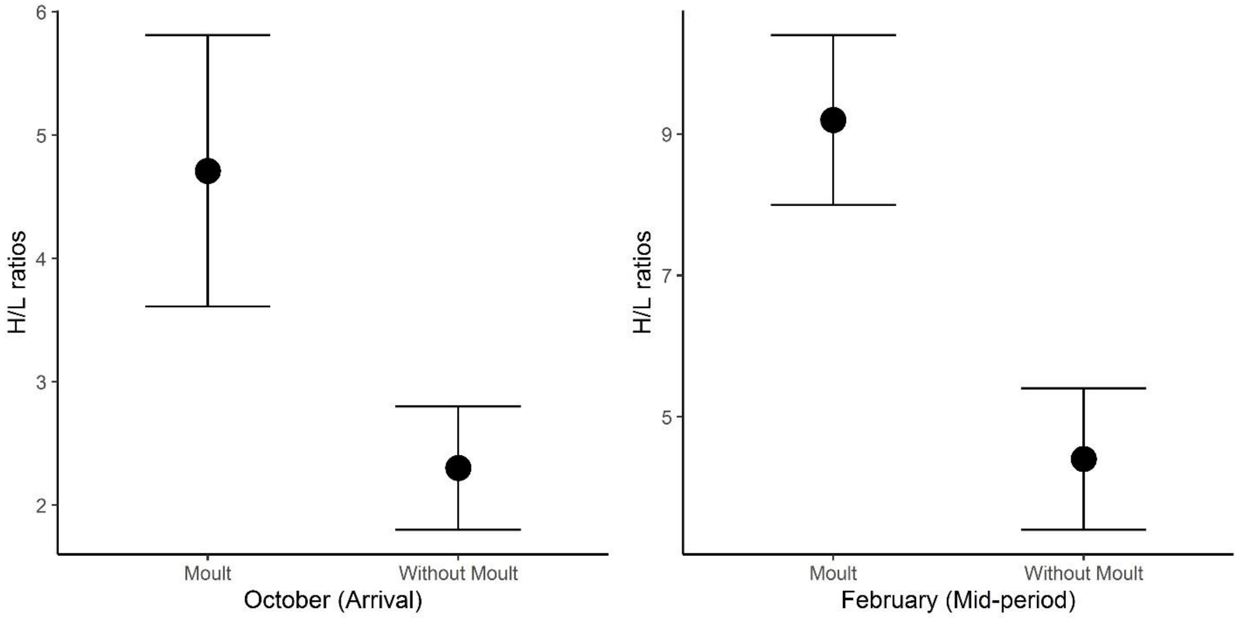
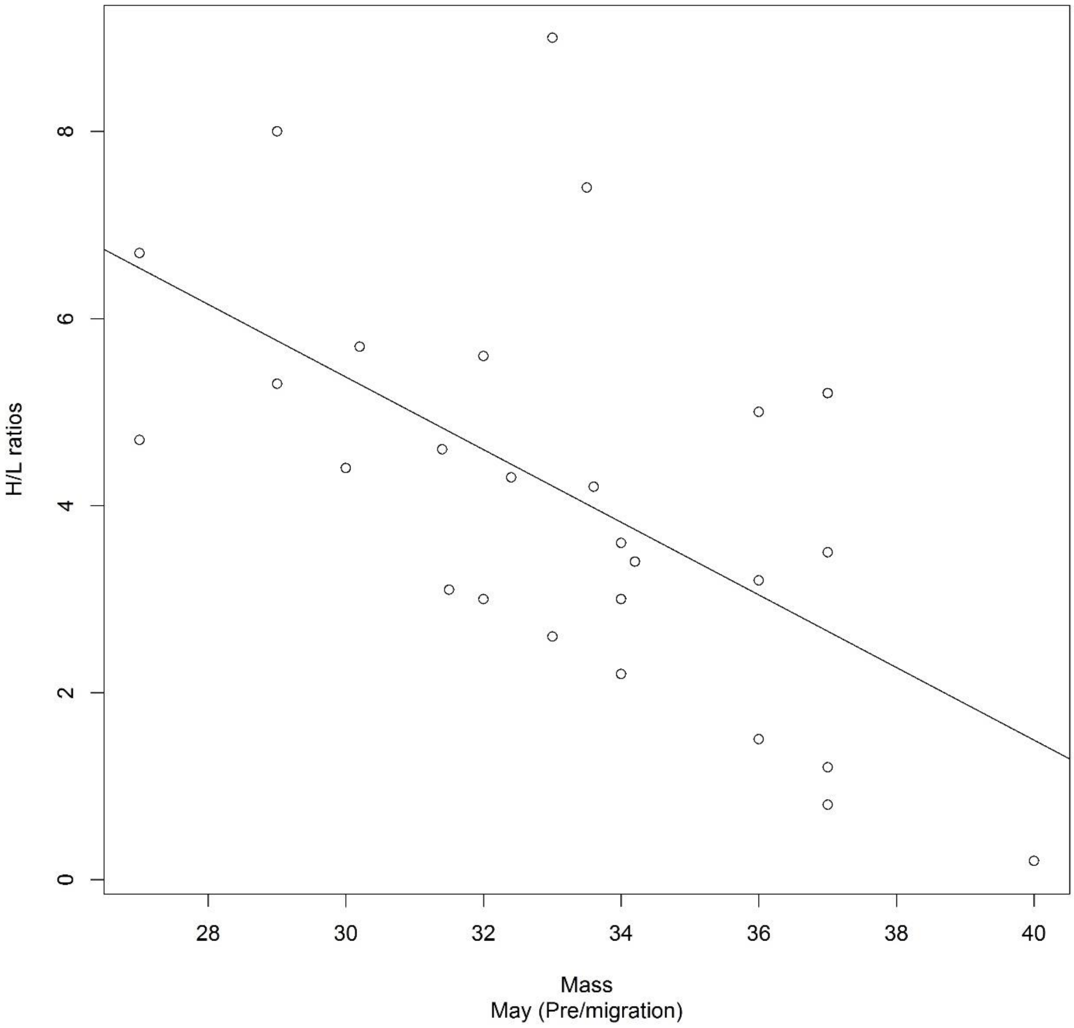


Fig. 3. Relationship between body mass and H/L ratios of Semipalmated Sandpiper in May (preparation to the north migration) at wintering sites on the Northeast coast of South America.



DISCUSSION

We investigated changes in the H/L ratio of Semipalmated Sandpiper in association with molt and body mass during its wintering in South America. Our results show that H/L ratios were positively associated with the presence of molt during arrival and the mid-wintering period. However, a negative relationship was found between the H/L ratio and body mass during preparation for the return migration to the northern hemisphere. These results support both of our hypotheses.

The molt process in birds is related to high energy demand (Piersma & Van Gils 2011, Alfaro et al. 2018), and, apparently, replacement of feathers is one of the main physiological stressors of Semipalmated Sandpiper during its wintering in the northeast of South America. Substantial increases in the metabolic rate occur during molting, when new body contour feathers are synthesized, and indirect costs can occur, e.g. an increase in thermoregulation costs associated with a decrease in plumage isolation (Buehler & Piersma 2008). Beside of that, additional metabolic processes have been recorded as flight feather substitutions due to higher maneuvers during the risk of predation or daily displacements for foraging, and this leads indirect energy cost (Hedenström 2003).

Lower H/L ratios have been correlated with higher body masses in several migratory birds, and this have been related to excellent nutritional status (Suorsa et al. 2004, Gladbach et al. 2010, Krams et al. 2011, Jakubas et al. 2015). Our results support this observation and suggest that the process of rapid mass gain reported during the pre-migratory period of Nearctic birds (Piersma et al. 1999, Battley & Piersma 2005) helps decrease the physiological stress of *C. pusilla* in order to begin its migration back to the northern hemisphere. Some bird species may respond to increases in body mass by decreasing physiological stress, as has recently been demonstrated in Common Snipe (Włodarczyk et al. 2018). Furthermore, the authors found that poor body condition increased stress levels and could compromise the annual cycle of the species (Włodarczyk et al. 2018).

In summary, our study indicates that the energy demand for plumage exchange of Semipalmated Sandpiper during the wintering period is associated with high physiological stress (H/L ratio). In general, the molting process determined the physiological stress levels of the species during most of wintering period until its preparation for the migration to the north when all birds concluded molting activities. However, we found a negative relationship between body mass and the H/L ratio, suggesting that mass gain during the final period of wintering in South America helps decrease physiological stress in Semipalmated Sandpiper.

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REFERENCES

Alfaro M., Sandercock B. K., Liguori L., Arim M. 2018. Body condition and feather molt of a migratory shorebird during the non-breeding season. Journal of Avian Biology 49(4): e. 01480.

Bates D., Maechler M., Bolker B., Walker S., Christensen R. H. B., Singmann H., Green P. 2018. Package ‘lme4’. Version. 1:17.

Battley P. F., Piersma T. 2005. Body composition and flight ranges of bar-tailed godwits (*Limosa lapponica baueri*) from New Zealand. The Auk. 122. 922-937.

Brown S., Jordan H. 2014. First ever geolocator results Semipalmated Sandpiper show remarkable yearlong odyssey. *In*: <https://www.manomet.org/newsletter/ first-ever-geolocator-results-semipalmated-sandpiper-show-remarkable-year-longodyssey>. Acesso em: 30 setembro 2018.

Buehler D. M., Piersma T. 2008. Travelling on a budget: predictions and ecological evidence for bottlenecks in the annual cycle of long-distance migrants. Philosophical Transactions of The Royal Society B: Biological Sciences 363: 247-266.

Cardoso T. A. L., Zeppelini D. 2011. Migratory shorebirds during boreal summer and southward migration on the coast of Paraíba, Brazil. Waterbirds 34: 369−375.

Cornelius E. A., Vézina F., Regimbald L., Hallot F., Petit M., Love O. P., Karasov W. H. 2017. Chickadees faced with unpredictable food increase fat reserves but certain components of their immune function decline. Physiological and Biochemical Zoology 90: 190–200.

Davis A. K. 2005. Effects of handling time and repeated sampling on avian white blood cell counts. Journal of Field Ornithology 76: 334–338.

Davis A. K., Cook K. C., Altizer S. 2004. Leukocyte profiles in wild house finches with and without mycoplasmal conjunctivitis, a recently emerged bacterial disease. EcoHealth 1: 362−373.

Davis A. K., Maney D. L., Maerz J. C. 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Functional Ecology 22: 760−772.

Eeva T., Hasselquist D., Langefors Å., Tummeleht L., Nikinmaa M., Ilmonen P. 2005. Pollution related effects on immune function and stress in free-living population of pied flycatcher *Ficedula hypoleuca*. Journal of Avian Biology 36:405−412.

Fokidis H. B., Greiner E. C., Deviche P. 2008. Interspecific variation in avian blood parasites and haematology associated with urbanization in a desert habitat. Journal of Avian Biology 39: 300−310.

Ginn H. B., Melville DS (eds). 1983. Moult in birds. Vol. 19. B.T.O. Guide.

Gladbach A., Gladbach D. J., Quillfeldt P. 2010. Variations in leucocyte profiles and plasma biochemistry are related to different aspects of parental investment in male and female upland geese *Chloephaga picta leucoptera*. Comparative Biochemistry and Physiology Part a Molecular & Integrative Physiology 156: 269–277.

Hedenström A. 2003. Flying with holey wings. Journal of Avian Biology 34:324–327.

Hinam H. L., Clair S. 2008. High levels of habitat loss and fragmentation limit reproductive success by reducing home range size and provisioning rates of Northern saw-whet owls. Biological Conservation 141:524−535.

Hully P., Craig A., Underhill G. 2004. Timing of moult and breeding in the Cape White-eye, *Zosterops pallidus*, from three different geographical regions in South Africa. Emu. 104: 353−358.

Ilmonen P., Hassequist D., Langefors Å., Wiehn J. 2003. Stress, immunocompetence and leukocyte profiles of pied flycatchers in relation to brood size manipulation. Oecologia 136:148−154.

Jakubas D., Wojczulanis K., Kośmicka A. 2015. Factors affecting leucocyte profiles in the little auk, a small Arctic seabird. Journal of Ornithology 156: 101–111.

Johnstone P., Richard D. R., Alan L. 2012. Interpreting indices of physiological stress in free-living vertebrates. Journal of Comparative Physiology 182: 861−879.

Krams I., Cīrule D., Krama T., Vrublevska J. 2011. Extremely low ambient temperature affects haematological parameters and body condition in wintering Great Tits (*Parus major*). Journal of Ornithology 152: 889–895.

Lindström Å., Visser G. H., Daan S. 1993. The energetic cost of feather synthesis is proportional to basal metabolic rate. Physiological zoology 66: 490–510.

Lobato E., Moreno J., Merino S., Sanz J. J., Arriero E. 2005. Haematological variables are good predictors of recruitment in nestling pied flycatchers (*Ficedula hypoleuca*). EcoScience 12:27−34.

Minias P., Kaczmarek K., Włodarczyk R., Janiszewski T. 2010. Adjustments and exploitation of body stores during post-juvenile molt in the common snipe *Gallinago gallinago*. Waterbirds 33:116–120.

Minias P., Meissner W., Włodarczyk R., Ożarowska A., Piasecka A., Kaczmarek K., Janiszewski T. 2015. Wing shape and migration in shorebirds: a comparative study. Ibis 157:528–535.

Minias P., Włodarczyk R., Meissner W. 2017. Leukocyte profiles are associated with longevity and survival, but not migratory effort: A comparative analysis of shorebirds. Functional Ecology 32:369-378.

Mizrahi D. S., Peters K. A., Hodgetts P.A. 2012. Energetic condition of Semipalmated and Least Sandpipers during northbound migration staging periods in Delaware Bay. Waterbirds 35: 135-145.

Morrison R. I. G. 2006. Body transformations, condition, and survival in red knots *Calidris canutus* travelling to breed at Alert, Ellesmere Island, Canada. Ardea 94:607-618.

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

Morrison R. I. G., Mizrahi R. K., Ross O. H., Ottema N., De Praconntal., Narine A. 2012. Dramatic declines of Semipalmated Sandpiper on their major wintering areas in the Guianas, northern South America. Waterbirds 35:120-134.

Müller C., Jenni-Eiermann S., Jenni L. 2011. Heterophils/Lymphocytes - ratio and circulating corticosterone do not indicate the same stress imposed on *Eurasian kestrel* nestlings. Functional Ecology 25. 566–576.

Murphy M. E., Taruscio T.G. 1995. Sparrows increase their rates of tissue and whole-body protein synthesis during the annual moult. Comparative Biochemistry and Physiology Part A: Physiology 111:385–396.

Owen J. C., Moore F. R. 2006. Seasonal differences in immunological condition of three species of thrushes. Condor 108: 389-398.

Pap P. L., Vágási C. I., Tökölyi J., Czirják G. Á., Barta Z. 2010. Variation in haematological indices and immune function during the annual cycle in the Great Tit *Parus major*. Ardea 98: 105–112.

Piersma T., Gudmundsson G. A., Lilliendahl K. 1999. Rapid changes in the size of different functional organ and muscle groups during refuelling in a long-distance migrating shorebird. Physiological and Biochemical Zoology 72:405-415.

Piersma T., Van Gils (eds). 2011. The flexible phenotype. A body-centered integration of ecology, physiology and behaviour. Vol.1. Oxford Univ. Press.

Rodrigues A. A. F. 2000. Seazonal abundance of neartic shorebirds in the gulf of Maranhão, Brazil. Journal of Field Ornithology 71: 665-675.

Rodrigues R. C., Azevedo Júnior, S. M., Larrazabal M.E.L., Araujo H. F. 2009. Temporal variations of body mass and plumage in *Arenaria interpres* (Aves, Scolopacidae) along the Brazilian coast. Zoologia 26:386-390.

Rodrigues R. C., de Araujo H. F. P., Guerra R. R., Durigon E. L., Mizrahi D. S., Azevedo Junior S. M. 2016. Temporal variation in the mass and plumage of four Charadriiformes species on the North-Eastern coast of Brazil. Emu 116: 461-466.

Suorsa P., Helle H., Koivunen V., Huhta E., Nikula A., Hakkarainen H. 2004. Effects of forest patch size on physiological stress and immunocompetence in an area-sensitive passerine, the Eurasian treecreeper (*Certhia familiaris*): an experiment. Proceedings of the Royal Society B: Biological Sciences 271:435−440.

Wikelski M., Cooke S. J. 2006. Conservation physiology. Trends in Ecology and Evolution. 21:38-46.

Włodarczyk R., Podlaszczuk P., Kaczmarek K., Janiszewski T., Minias P. 2018. Leukocyte profiles indicate nutritional, but not moulting stress in a migratory shorebird, the Common Snipe (*Gallinago gallinago*). Journal of Ornithology 159: 345-354.

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CAPÍTULO 2

Comportamento oportunista e variação no investimento de forrageio de maçarico-rasteirinho (*Calidris pusilla*) durante inverno na América do Sul

AGOSTO/2021

Original articles

OPPORTUNIST BEHAVIOR AND VARIATION IN FORAGE INVESTMENT IN SEMIPALMATED SANDPIPER (*Calidris pusilla*) DURING WINTER IN SOUTH AMERICA

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OPPORTUNIST BEHAVIOR AND VARIATION IN FORAGE INVESTMENT IN SEMIPALMATED SANDPIPER (*Calidris pusilla*) DURING WINTER IN SOUTH AMERICA

ABSTRACT

Every year, thousands of shorebirds migrate to wintering areas in South America. In these areas, they feed and store energy for various physiological processes. To supply the high energy demand for successful migration, it is essential that these birds have the ability to forage for a variety of prey. In this study, we analysed whether the foraging behaviour and diet of the Semipalmated Sandpiper (*Calidris pusilla*) varied during winter in South America. For this, we tested two hypotheses: (1) The bird increases its foraging investment before its migration back to the northern hemisphere. If this happens, we would expect an increase in foraging investment to occur regardless of the variation in available prey. (2) The Semipalmated Sandpiper will present opportunistic behaviour as a foraging strategy to supply its energy needs throughout the wintering period. Thus, we expect that there will be a positive association between the foraging method and the prey that is most available for food. This study was carried out during the winter stay of Semipalmated Sandpiper in the northeast of South America, where we recorded individuals during the low tide in the Rio Paraíba estuary. In summary, we verified that the Semipalmated Sandpiper can vary its foraging investment regardless of prey variation. Semipalmated Sandpiper also displayed opportunistic behaviours in foraging.

KEYWORDS

Charadriiformes, foraging strategy, macroinvertebrates, migration, shorebirds

INTRODUCTION

Every year, thousands of shorebirds migrate seasonally from their breeding sites north of the Tropic of Cancer to winter in tropical and subtropical regions (Harrington & Morrison, 1979; Newton, 2007). In these areas, birds feed and store the energy necessary for physiological processes, such as feather moults, which are essential for flight and the accumulation of sufficient mass to return to breeding sites (Placyk & Harrington, 2004; Rodrigues et al., 2009). To supply this high energy demand, it is essential that the birds are able to adjust their foraging variation, ensuring successful migration (Martins et al., 2013).

The flexibility that shorebirds present with foraging behaviour may be associated with prey choice (Mailleit & Weber, 2006; McDonald et al., 2012). A varied diet in birds with long migrations, such as the Semipalmated Sandpiper (*Calidris pusilla*), includes gastropods, bivalves, crustaceans and polychaetes, and opportunistic foraging for this bird has been suggested (Quin & Hamilton, 2012; Skagen & Oman, 1996). Semipalmated Sandpiper feed primarily by skimming their beaks across a surface rather than the typical pecking and probing they exhibit at stopover sites when populations of *Corophium volutador* are low and Ostracoda high (McDonald et al., 2012). There is a hypothesis that foraging strategies can optimize the life stages of these birds and that hyperphagic behaviour is used to acquire energy for the physiological processes of the migratory cycle (Berthold, 1975).

Semipalmated Sandpiper arrive on the northeast coast of South America after long migratory flights, and for approximately 200 days, they face different energy demands (Rodrigues et al., 2016). For example, there is a positive relationship between stress indicators and moult activity during this wintering period but a negative relationship between stress indicators and the accumulation of body mass to start returning to the northern hemisphere (Silva et al., 2021). Thus, this is an excellent opportunity to verify variations in the foraging investment and diet of Semipalmated Sandpiper during the annual cycle at wintering sites.

We aimed to analyse whether the foraging behaviour and diet of the Semipalmated Sandpiper varied during wintering in South America. We tested two hypotheses: (1) The bird increases the time invested in the foraging method in order to supply the energy demand for the period of return to the northern hemisphere. If this happens, we expect an increase in foraging investment regardless of the variation in available prey. (2) The Semipalmated Sandpiper will present opportunistic behaviours as a foraging strategy to supply its energy needs for the entire wintering period. Thus, we expect a positive association between the foraging method and the most abundant prey. With that, we intend to fill a knowledge gap in the migration ecology of the Semipalmated Sandpiper, demonstrating how the foraging method can vary during different phases of its winter period in South America.

METHODOLOGY

Study area

We conducted this study in November, February of 2017 and May of the 2018 during the winter period of the Semipalmated Sandpiper on Restinga Island, located in the Rio Paraíba estuary in northeast South America (6°59′6″ S, 34°51′52″ W). This river island consists mainly of secondary rainforests (the Atlantic Forest) and mangroves. The main foraging area of the Semipalmated Sandpiper on the island is an extensive mud/sandbank that forms with water recession (6°58′50″ S, 34°51′30″ W; Cardoso & Zepellini, 2011).

Sampling protocol

We recorded Semipalmated Sandpiper individuals randomly during the low tide (± 2 h before/after peak tide) in the wintering period in the study area. We used a digital camera (Nikon Coolpix P900; Nikon, Tokyo, Japan) with 83x optical zoom mounted on a tripod approximately 2 metres above the ground for the recordings. We filmed the birds at short distances of 15 to 30 metres. As the tidal recession is relatively quick, mudflats are constantly exposed, so the Semipalmated Sandpiper often chooses their foraging areas and feeds in the same direction. Thus, pseudoreplication (filming the same bird more than once) is unlikely to occur for the *Calidris* genera (Martins et al., 2013). This bird feeds in the study area with two methods that have been well described in the literature for other areas: pecking, which is a shallow penetration of the bill into the sediment, and probing, which is a deeper penetration of the bill into the sediment (Baker & Baker, 1973).

A total of 51 one-min videos were recorded. To analyse the time invested in foraging and the prey consumed, we followed the protocol of Martins et al. (2013). We quantified the number of pecks/min and the prey consumed/min for each foraging method used by the Semipalmated Sandpiper. For the characterization of the diet, we quantified the prey consumed as filiform or non-filiform. Filiform prey (e.g. polychaetes) are easily identified (Martins et al., 2013). However, non-filiform prey (e.g. crustaceans, gastropods and bivalves) are challenging to visualize in the slow-motion video and are thus usually identified with typical swallowing movements (Martins et al., 2013; Santos et al., 2010).

Prey availability

The density of potential prey was analysed with sediment samples (collected in 100 cm^2 of the area at 5 cm depth). It was sieved in situ with a 0.2-mm mesh and was fixed in 10% formalin. For sampling, we marked 10 points at equal distances along with the transaction. We gathered two sediment samples from these points, one on the right and one on the left of each point. In November, February and May, we collected 20 sediment samples. In the lab, we identified and quantified the organisms with a stereoscopic microscope and identified them up to class (due to the limited identification of our videos). The protocol used to identify macroinvertebrates was from Amaral et al. (2006). The macroinvertebrate count was used to estimate the number of macroinvertebrates/m² (Hicklin & Smith, 1984).

Statistical analysis

To analyse the differences during the months of this research (November, February and May) for the surface availability variable (individuals/m²) and the macroinvertebrate classes (gastropods and bivalves), we used one-factor ANOVA with a significance of 5% ($p < 0.05$) after we found normality and heterogeneity.

We verified the differences between the months of number of pecks/min with the foraging style (pecking or probing) using Kruskal–Wallis (KW) and Dunn post hoc (*df*) tests with multiple comparisons conducted, both with a significance of 5% ($p < 0.05$). Similarly, for the prey consumed/min with foraging pecking or probing styles, we analysed the differences between the months using the Kruskal–Wallis and Dunn post hoc tests, with a significance of 5% ($p < 0.05$).

To analyse the dependence of the foraging style, pecking or probing, on non-filiform and filiform prey, we used generalized linear models (GLM) adjusted to the Gaussian distribution, with a significance of 5% ($p < 0.05$). The GLM analysis was performed using the statistical package for the R platform, version 3.5.0 (linear and nonlinear mixed-effects models; Bates & Pinheiro, 2018). The R platform, version 3.5.0, was also used for all other analyses.

RESULTS

During the wintering period of the Semipalmated Sandpiper, the surface prey with the highest average abundance were gastropods and bivalves (Table 1). However, we did not find significant differences in macroinvertebrates between the months analysed (Table 1, Figure 1a).

In contrast, significant differences were observed for the investment in the number of Semipalmated Sandpiper' pecks/min with the foraging styles of pecking or probing in the same period (KW = 37.08, *df* = 5, *p* = 0.0000005; Figure 1b). We observed variations between pecking and probing for the months of November (*p* = 0.01), February (*p* = 0.0005) and May (*p* = 0.002), with the highest averages for pecking (Figure 1b). Pecking and probing varied from November to February (pecking, *p* = 0.002; probing, *p* = 0.029) and November to May (pecking, *p* = 0.006; probing, *p* = 0.032), but not from February to May (pecking, *p* = 0.3716; probing, *p* = 0.4926; Figure 1b).

We found significant variations in prey consumed/min with the analysed foraging behaviours for all months (KW = 42.91, *df* = 5, *p* = 0.00000003; Figure 1c), with variations in the effectiveness of prey consumed/min between pecking and probing for Nov (*p* = 0.0005), Feb (*p* = 0.00001) and May (*p* = 0.0005; Figure 1c). The highest averages of prey consumed/min using pecking and probing behaviours were for February (Figure 1c). We observed variations in the effectiveness of prey consumed/min with pecking behaviour only between the months of November and February (pecking, *p* = 0.002) and November and May (pecking, *p* = 0.0118; Figure 1c). However, the effectiveness of prey consumed with the probing behaviour did not vary between the months of November and February (*p* = 0.0877), November and May (*p* = 0.1614) and February and May (*p* = 0. 3662; Figure 2c).

We found a significant association between pecking and foraging behaviour during all months with non-filiform prey (Table 2). All pecking and non-filiform prey relationships were positive (Figure 2). In contrast to these results, we found no significant relationships between probing behaviour and filiform or non-filiform prey (Table 2, Figure 2).

TABLE 1. Seasonal variations in the count of macroinvertebrates consumed by *Calidris pusilla* during the winter (November, February and April) on Restinga Island in northeast South America

Surface availability (individual/m^2)	**Nov**	**Feb**	**May**	**KW or F**	***P*-value**
Seasonal					
Gastropod	296.0	289.8	287.6	F: 0.021	0.979
Bivalve	132.8	131.4	130.1	F: 0.004	0.996
Polychaete	2.2	1.4	1.6	KW: 0.948	0.622
Crustacean	0.35	0.5	0.2	KW: 0.214	0.898
Total availability	431.4	423.3	419.6	F: 0.032	0.968

Abbreviations: F, distribution of Dunn’s post hoc test; KW, Kruskal–Wallis.

TABLE 2. Generalized linear models between non-filiform prey and pecking or probing behaviour and filiform prey and pecking or probing behaviour of *Calidris pusilla* during the wintering period in South America

Predictors	**T**	***P*-value**	***B***
Nov			
Non-filiform prey with pecking	2.488	< **0.05***	6.964
Filiform prey with pecking	−1.061	0.305	−0.001
Non-filiform prey with probing	−0.304	0.765	−0.005
Filiform prey with probing	−0.190	0.851	−0.003
Feb			
Non-filiform prey with pecking	0.019	< **0.05***	0.140
Filiform prey with pecking	0.635	0.534	0.001
Non-filiform prey with probing	0.272	0.789	0.003
Filiform prey with probing	0.290	0.775	0.005
May			
Non-filiform prey with pecking	4.435	< **0.05***	0.157
Filiform prey with pecking	−0.671	0.513	−0.001
Non-filiform prey with probing	0.767	0.546	0.006
Filiform prey with probing	1.582	0.136	0.032

Abbreviations: *B*, Estimate; T, t value.

***** Significance at Nov, p-value 0.0251; Feb, p-valuer 0.000002; May, p-valuer 0.0005

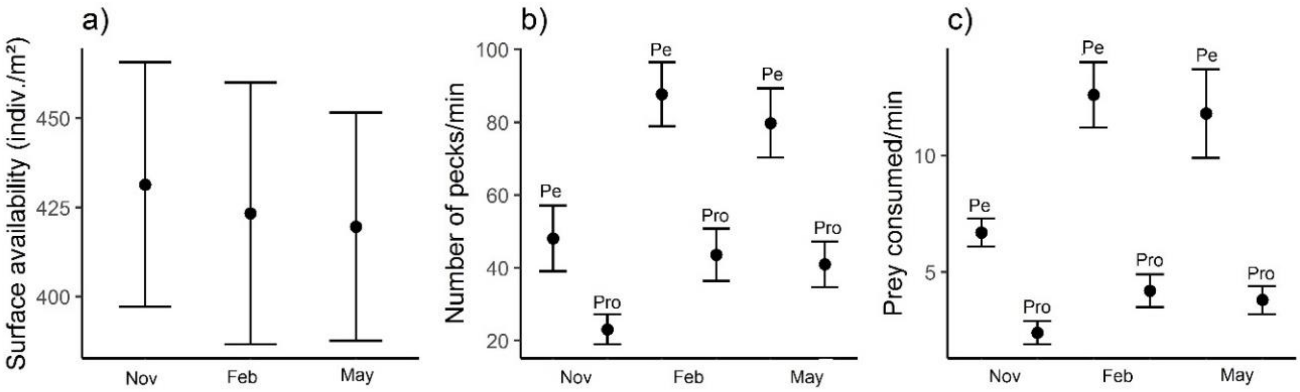

FIGURE 1. Variation of surface availability (individual/m²), number of pecks/min, and prey consumed/min by *Calidris pusilla* during November, February and May on Restinga Island in northeast South America

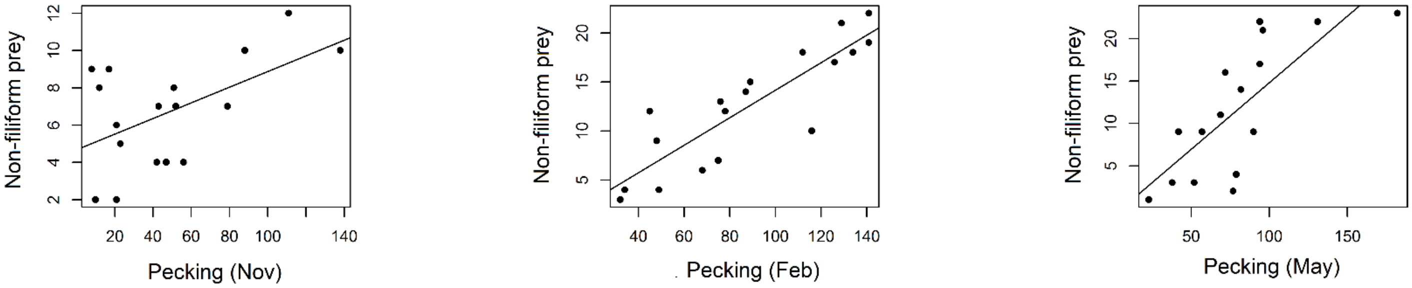

FIGURE 2. Linear regressions between pecking or probing behaviour and non-filiform prey and pecking or probing behaviour and filiform prey of *Calidris pusilla* during winter months (November, February and May) on Restinga Island in northeast South America

DISCUSSION

We investigated changes in the variation of foraging behaviour and diet of Semipalmated Sandpiper during their wintering period on the northeast coast of South America. We found that the periods with the greatest investments in foraging behaviour and the highest number of prey consumed were the months of February and May. Furthermore, we observed a positive relationship between the foraging behaviour of pecking and non-filiform prey, which was the most available prey. This indicates that opportunistic behaviour was exhibited by the birds during the winter.

Some studies have suggested that hyperphagic behavioural adjustments in migratory birds are the first response when there is an increase in metabolic demands because these adjustments are faster and cheaper than physiological mechanisms (Guillemette, 2001, 2012). A significant increase in Semipalmated Sandpiper food searching during February and May, as seen here, is possibly related to increased metabolic demand. During the wintering of shorebirds on the northeast coast of South America, the mid-period, which takes place in February, presents high levels of energy expenditure for processes that include changes in flight and contour feathers (Rodrigues et al., 2016; Silva et al., 2021). Furthermore, a significant increase in the depth of intestinal crypts during the middle of winter has been recorded in Semipalmated Sandpiper (Chaves et al., 2018). This possibly indicates that the Semipalmated Sandpiper is increasing its weight in the final phase of winter as a physiological preparation for the May migration. According to Moreira-Filho et al. (2015), the increase in the depth of intestinal crypts is related to the increase in the area of the intestinal epithelium in contact with ingested food, thus absorbing more nutrients. The pattern of higher investment in Semipalmated Sandpiper foraging with pecking and probing behaviours in the middle and end of winter, along with the higher number of prey consumed/min, is possibly an adjustment to meet the energy demand of this period.

Opportunistic foraging in shorebirds is an adaptation to fit the availability of macroinvertebrates at a stopover site. They feed mainly on more abundant prey to acquire the energy necessary for the high metabolic demand of completing the migratory cycle (Brenan et al., 1990; Davis & Smith, 2001; Mathot et al., 2010; McDonald et al., 2012; Smart & Gill, 2003). At stopover sites in the southern Great Plains, American avocets (*American recurvirotra*) and long-billed dowitchers (*Limnodromus scolopaceus*), which are large shorebirds capable of handling more profitable prey, choose smaller prey because they are more abundant (Davis & Smith, 2001). These results seem to agree with the observations in the present study of the Semipalmated Sandpiper, which performed the pecking foraging method more frequently and probing less frequently due to the large abundance of non-filiform prey throughout the winter. This pattern has also been observed in dunlins' (*Calidris alpina*) consumption of non-filiform prey, such as *Hydrobia ulvae* and siphons of *Flat scrobicularia,* in a south European estuary (Martins et al., 2013). These patterns are possibly used by *Calidris* to optimize foraging due to visually based strategies increasing the encounter rate and the success of capturing prey on the surface (Zwarts & Esselink, 1989).

In summary, our study indicates that the Semipalmated Sandpiper varied its foraging investment, possibly to adjust for energy demands, in a wintering area in South America. In addition, this bird exhibited opportunistic behaviours by feeding on the most readily available prey. These results help build an understanding that the Semipalmated Sandpiper can vary its foraging behaviour in the wintering areas of South America, using different foraging strategies to supply its energy needs for the migratory cycle.

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REFERENCES

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, RHB, Singmann, H., Green, P. (2018). Package ‘lme4’. Version. 1:17.

Berthold, P. (1975). Migration: control and metabolic physiology. Em JR King (Ed.), Avian Biology, Vol.1 (pp.77-128). New York: Academic Press.

Brennan, LA, Finger, MA, Buchanan, JB, Schick, CT, & Herman, SG (1990). Stomach contents of Dunlins collected in western Washington. Northwestern Naturalist, 71, 99–102. doi.org/10.2307/3536782

Chaves, FQ, Neri-Júnior, NA, Rodrigues, RC, Araujo, HF, & Guerra, RR (2018). Morphometry of pectoral muscle fiber and intestinal villi of *Calidris pusilla* during the wintering period in Brazil. Pesquisa Veterinária Brasileira, 9, 1849-1855. doi.org/10.1590/1678-5150-PVB-5175

da Silva, LSA, Rafael, RM, Rodrigues, GG, de Araujo, HP (2021). Heterophile/lymphocyte profiles are associated with mass increase and moulting in the Semipalmated Sandpiper *Calidris pusilla* at wintering sites in NE South America. Acta Ornithol. 56.

Davis, CA, & Smith, LM (2001). Foraging strategies and niche dynamics of coexisting shorebirds at stopover sites in the southern Great Plains. Auk, 118, 484–495. doi:10.1642/0004-8038(2001)118[0484;FSANDO]2.0.CO;2

Guillemette, M, Richman, SE, Portugal, SJ, & Butler, PJ (2012). Behavioural compensation reduces energy expenditure during migration hyperphagia in a large bird. Functional Ecology, 26, 876-883. https://doi.org/10.1111/j.1365-2435.2012.01993.x

Guillemette, M., (2001). Foraging before spring migration and before breeding in Common Eiders: does hyperphagia occur? Condor, 103, 633– 638. https://doi.org/10.1093/condor/103.3.633

Harrington, BA, & Morrison, RIG (1979). Semipalmated Sandpiper (*Calidris pusilla*). Wader Study Group Bulletin, 33, 37-38.

MacDonald, EC, Ginn, MG, & Hamilton, DJ (2012). Variability in foraging behaviour and implications for diet breadth among Semipalmated Sandpiper staging in the upper Bay of Fundy. Condor, 114, 135-144. doi.org/10.1525/cond.2012.100246

Maillet, D, & Weber, JM (2006). Performance-enhancing role of dietary fatty acids in a long-distant migrant shorebird: the Semipalmated Sandpiper. Journal of Experimental Biolology, 14, 2686-2695. doi:10.1242/jeb.02299. PMID:16809459.

Martins, RC, Catry, T., Santos, CD, Palmeirim, JM, Granadeiro, JP (2013) Seasonal Variations in the Diet and Foraging Behaviour of Dunlins *Calidris alpina* in a South European Estuary: Improved Feeding Conditions for Northward Migrants. PloS one 8: e 81174, 2013. doi.org/10.1371/journal.pone.0081174

Moreira-Filho, ALB, Oliveira, CJB, Oliveira, HB, Campos, DB, Guerra, RR, Costa, FGP, & Givisiez, PEN (2015). High incubation temperature and threonine dietary level improve ileum response against post-hatch *Salmonella enteritidis* inoculation in broiler chicks. Plos One 10:e0131474. doi.org/10.1371/journal.pone.0131474

Newton, I. (2007). The migration ecology of birds. London: Academic Press.

Placyk, JJR, & Harrington, BA (2004). Prey abundance and habitat use by migratory shorebirds at coastal stopover sites in Connecticut. Journal of Field Ornithology 75, 223-231. doi: 10.1648/0273-8570(2004)075[0223:PAAHUB]2.0.CO;2

Quinn, JT, & Hamilton, DJ (2012) "Variation in diet of Semipalmated Sandpiper (*Calidris pusilla*) during stopover in the upper Bay of Fundy, Canada. Canadian Journal of Zoology, 90, 1181. doi.org/10.1139/z2012-086

.Rodrigues, RC, Araujo, HFP, Guerra, RR, Durigon, EL, Mizrahi, DS, & Azevedo-Junior, SM (2016). Temporal variation in the mass and plumage of four Charadriiformes species on the north-eastern coast of Brazil. Emu-Austral Ornithology, 116, 461-466. doi.org/10.1071/MU15083

Silva, LSA, Rafael, RM, Rodrigues, GG, & de Araujo, HP (2021). Heterophile/lymphocyte profiles are associated with mass increase and moulting in the Semipalmated Sandpiper *Calidris pusilla* at wintering sites in NE South America. Acta Ornithol, 56, 127–132.

Skagen, SK, & Oman, HD (1996). Dietary flexibility of shorebirds in the western hemisphere. The Canadian Field-Naturalist. 110, 419-444.

Smart, J., & Gill, JA (2003). Non-intertidal habitat use by shorebirds: a reflection of inadequate intertidal resources? Biological Conservation 111, 359-369. doi:10.1016/S0006-3207(02)00304-X

Zwarts, L., Esselink,. P. (1989). Versatility of male curlews *Numenius arquata* preying upon *Nereis diversicolor*- deploying contrasting capture modes dependent on prey availability. Marine Ecology-Progress Series, 56, 255–269.

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CAPITULO 3

Influência da variação de recursos alimentares no investimento de forrageio e indicadores de estresse de maçarico-rasteirinho (*Calidris pusilla*) durante o inverno

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Influence of food resource variation on foraging investment and stress indicators of the Semipalmated Sandpiper (*Calidris pusilla*) during winter

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Influence of food resource variation on foraging investment and stress indicators of the Semipalmated Sandpiper (*Calidris pusilla*) during winter

Abstract

The Semipalmated Sandpiper are shorebirds known for making great flights during their annual cycle. They also present foraging strategies because of the significant variation of food resources, which impacts their physiological and nutritional status. In this study, we evaluate how the Semipalmated Sandpiper (*Calidris pusilla*) invests in foraging time during food variations in a South America wintering area. Additionally, we verify the monthly impact of food variation on the nutritional status and physiological stress indices of Semipalmated Sandpiper. A survey was carried out in Restinga Island, Brazil, which is located northeast of South America, in October, January and April for the years 2018 and 2019. Data collection was carried out at two selected points on Restinga Island, which were used as foraging and resting locations for Semipalmated Sandpiper. We found variations in prey availability (ind. per/m^2) and prey consumed per/min, with progressive reductions between months. Semipalmated Sandpiper varied its number of pecks/min, with a higher mean in January and a lower mean at the end of winter in April. Additionally, physiological stress indices showed significant variations, with the highest rates at the end of winter in April. Our study indicates that Semipalmated Sandpiper experience successive periods of food shortages and an increase in stress indicators during the winter period in South America. These results may help us understand the impact that food variations can have on migratory birds that need adequate habitats to supply the physiological demand of their migratory cycle.

Keywords: Charadriiformes, nutritional stress, macroinvertebrates.

Introduction

The Semipalmated Sandpiper (*Calidris pusilla*) is a shorebird that engages in long flights during seasonal cycles and needs adequate food to rebuild their energy reserves and to help supply a higher physiological demand during migration (Schaub et al. 2008). However, these birds need to deal with the challenge of finding prey in varied habitats and experience unfavourable biotic conditions that limit these choices (Piersma et al. 1993; Martins et al. 2013), which can compromise their migration (Morrison et al. 2004). This process takes place during the winter and has had noticeable records of population decline in recent years (Morrison et al. 2004, 2012).

The Semipalmated Sandpiper is one of those shorebirds found along the South American coast during the winter (Cardoso et al. 2013). These birds are abundant in estuarine areas, which are responsible for large amounts of macroinvertebrates, which are part of their diet (Fedrizzi et al. 2016). Although Semipalmated Sandpiper can feed on specific prey at essential sites in their migration (Hicklin and Smith 1979; Skagen and Oman 1996; Maillet and Weber 2006), they can also be generalists in other sites, foraging numerous species, such as polychaetes, insects, crustaceans, gastropods and bivalves (Quin and Hamilton 2012). With these strategies, shorebirds can alleviate food resource unpredictability, which can impact physiological states and affect responses to stress (Owen and Moore 2006).

Recently, it has been suggested that the combination of low energy reserves and insufficient food availability may cause a stressful environment, which in turn promotes responses in stress indicators (H/L ratio) for short-distance migration (Włodarczyk et al. 2018). An investigation into this migratory foraging process for Semipalmated Sandpiper may help us understand how birds of long-distance migrations circumvent these situations, as there is a negative association between nutrition and indicators of physiological stress of these birds during migration preparation (Silva et al. 2021).

This study investigated the impacts that food resource variation can cause on Semipalmated Sandpiper in a stopover area during the winter in northeast South America. Our first objective was to analyse how the investment of foraging Semipalmated Sandpiper adjusts due to the variation in prey availability. Additionally, our second objective determines the influence of prey's monthly unpredictability on variations of body conditions (mass) and indicators of physiological stress (H/L ratio). We expected that a variation in foraging investment could adjust the food available, benefit the nutrition and reduce physiological stress in a favourable environment. However, if food is limited, the foraging investment cannot promote the prey's high consumption, affecting nutrition and physiological stress. We hope to fill the knowledge gap in the Semipalmated Sandpiper migration ecology and to demonstrate how their physiological and behavioural processes are affected by dietary variations during their migratory cycle.

Methodology

Study area

Data collections were conducted in October, January and April 2018/2019 during the winter period in Restinga Island, located in the Rio Paraíba estuary, northeast of South America (6° 59' 6''S, 34°51'52''W) (Figure 1). This fluvial island has vegetation composed mainly of remnants of Atlantic Forest and mangroves. A temporal lake (6° 59' 12" S, 34 ° 51' 36" W) on Restinga Island, which measures approximately 700 m, is formed daily at high-tide and is where flocks of Semipalmated Sandpiper gather as a principal resting place (Cardoso and Zepellini 2011) (Figure 1). In northern Restinga Island, 200 m away from the principal resting place of Semipalmated Sandpiper, a mud/sandbank approximately 1.5 km long is formed in response to the low-tide (6° 58 '50' 'S, 34 ° 51' 30 ' 'W) (Figure 1), where large groups of Semipalmated Sandpiper can be seen foraging every day during the winter (Cardoso and Zepellini 2011).

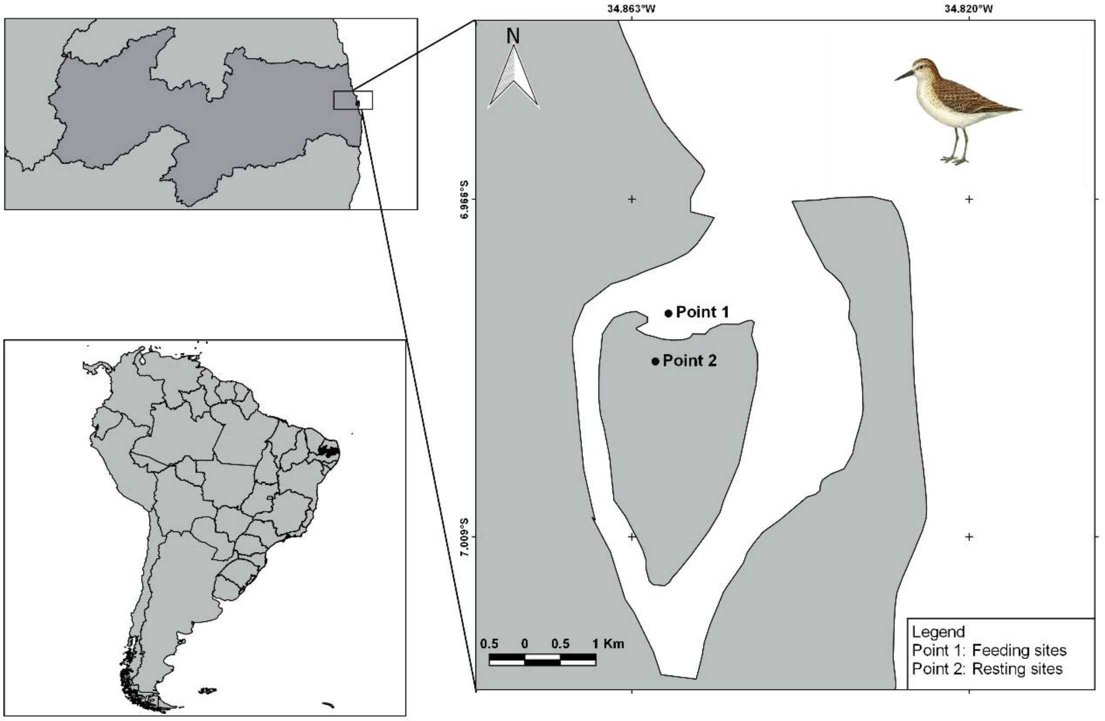

Figure 1. Map with Restinga island location, showing the 2 points used for foraging (point 1) and resting (point 2) of *Calidris pusilla* during the wintering in the northeast of South America.

Prey sampling and availability

The density of potential prey for our study was determined with sediment samples (collector: 100 cm^2 of the area and 5 cm in depth). The soil samples were established from a transect perpendicular to the waterline. For sampling purposes, we marked 10 points equidistant along with the transaction. We gathered two sediment samples from these points, one on the right and one on the left of each point. On a monthly basis, from

October, January and April, we collected 20 sediment samples. The samples were sieved 'in situ' with a 0.2 mm mesh, and the retained material was fixed in 10% formalin. In the lab, we used a stereoscopic microscope to screen, identify and quantify the organisms, which were identified only up to class (due to the limited identification of our videos), and preserved in 4% formaldehyde. The procedures for the identification of macroinvertebrates followed the protocols based on Amaral et al.'s 2006 study. The macroinvertebrate count was used to estimate the potential prey density, or in other words, the number of macroinvertebrates/m^2 (Hicklin and Smith 1984).

Foraging behavior and diet characterization/min

Video recordings recorded individual Semipalmated Sandpiper during the daily period of low-tide (± 2 h before/after peak tide) while the birds were foraging in the northern area of Restinga Island. These recordings were made with a digital camera (Nikon Coolpix P900) with an 83x optical zoom. We recorded the birds at a distance of approximately 40 to 50 m, in closed places, outside the vision of Semipalmated Sandpiper; we tried to avoid stress or changes in eating habits due to the researcher's presence (Cestari 2014). Flocks of the genus *Calidris* are generally numerous (100–200 individuals) and move in the same direction, decreasing the probability of pseudoreplication (filming the same bird more than once) (Martins et al. 2013). Semipalmated Sandpiper feed at the water's edge when the tide recedes (MacDonald et al. 2012); thus, we tried to wait for individual birds to choose their main point to start foraging, and we started filming to record the frequency of pecks/min and prey consumed/min.

We recorded filiform prey/min of macroinvertebrates (e.g., polychaetes), which are easily identified (Martins et al. 2013). The non-filiform prey/min (crustaceans, gastropods and bivalves) were recognized by typical swallowing movements, known for mollusks and crustaceans (Santos et al. 2010; Martins et al. 2013). For our data, we consider these macroinvertebrates (gastropods, bivalves, polychaetes and crustaceans) as prey to be consumed/min. Both classes of macroinvertebrates were viewed during one minute of the recording (MacDonald et al. 2012; Martins et al. 2013) for each individual Semipalmated Sandpiper in October, January and April.

Catching birds and Blood smears

After the recording stage and prey sampling (at low-tide), the Semipalmated Sandpiper flocks moved to the main lake to rest, which was at high-tide (Figure 1). At this moment, we caught some birds using mist nets. For body mass, dynamometer-type scales with an accuracy of 1 g were used, as molting affected the stress indicators (H/L ratio) in Semipalmated Sandpiper (Silva et al. 2021). We did not use data from individual birds with any evidence of molting.

We obtained the concentrations of physiological stress indicators through indicators through the blood of the brachial vein located in their wing. Smears were prepared on blades and stained using the May–Grunwald–Giemsa (MGG) method. The blades went to the lab, where the immunological rates of heterophile counts by lymphocytes (H/L ratio) were generated through microscopic observations. The variation of these rates can be used to indicate changes in physiological stress regarding the biotic variables (availability of food resources) responsible for supplying the animal’s energy demand (Johnstone et al. 2012). The collection of blood samples was the first activity of the capture procedure, which reduced the possibility of changes in the blood parameters influenced by possible stress due to capture (Johntone et al. 2012).

Statistical analysis

To verify the difference during the months of the study period (October, January and April) regarding the total prey and each class of macroinvertebrates, we used the

ANOVA 1-Factor and Post-Hoc Tukey tests, with 5% significance ($p < 0.05$) after following the assumptions of normality and heterogeneity.

To analyse the difference of each variable (1-number of pecks/min, 2-prey consumed/min, 3-stress indicators [H/L rates] and bivalve density [per m^2]) among the time cycle (October, January and April), we used the Kruskal-Wallis and Post-Hoc tests with multiple comparisons conducted, both with a significance of 5% ($p < 0.05$).

To analyse the dependence on the number of pecks/min concerning the prey consumed/min for each month in the winter cycle (October, January and April), we used Generalized Linear Models (GLM), adjusted to the Gaussian distribution, with a significance of 5% ($p < 0.05$).

Results

We found significant variations in prey availability (per m^2) between the seasonal cycle of this survey ($F = 9.442$, $p = 0.0002$) (Figure 2a), with variations between October and January ($p = 0.008$) and October and April ($p = 0.0002$). We did not, however, find differences between January and April ($p = 0.5094$) (Figure 2a). Although, there was a decrease in prey availability (per m^2) among the months of this survey: October (± SE= 444.5), January (± SE= 281.5) and April (± SE= 222.6) (Figure 2a). A reduction in gastropods and bivalves was found over the winter months, with the highest averages in October being gastropods (± SE= 307.6) and bivalves (± SE= 134.3), and the smallest averages in April being gastropods (± SE= 145.3) and bivalves (± SE= 51.9) (Table 1). However, we did not find a significant difference for polychaetes (± SE= 1.8, $F = 0.385$, $p = 0.682$) or crustaceans (± SE= 0.2, $F = 0.135$, $p = 0.874$) (Table 1) among the months.

The number of pecks of the foraging Semipalmated Sandpiper in the 85 recorded videos analysed during the winter (October, January and April) varied significantly: KW = 9.801, p = 0.007 (Figure 2b). Differences occurred between October and April (p = 0.017) and January and April (p = 0.001); however, we did not find differences between October and January (p = 0.122) (Figure 2b). The highest average number of pecks of the foraging Semipalmated Sandpiper was registered in January (± SE= 124.4), whereas the lowest number was registered in April (± SE= 66.7) (Figure 2b). Although this seemed to be a positive trend, we did not find significant effects of frequency regarding pecking/min on prey consumed/min during the months of October (T = 1.554, p = 0.130), January (T = 0.092, p = 0.927) or April (T = 1.175, p = 0.250) (Figure 3).

The prey consumed/min by Semipalmated Sandpiper during the winter period varied significantly (KS = 31.259, p < 0.001), with a progressive decrease during the winter cycle: October (± SE= 9.3), January (± SE= 4.3) and April (± SE = 2) (Figure 4a). We found significant differences in prey consumed per/min among all months: October and January (p = 0.04), October and April (p < 0.001) and January and April (p < 0.001) (Figure 4a). There was a progressive decrease in the body mass of Semipalmated Sandpiper between the months of October (± SE= 24.4), January (± SE= 23.2) and April (± SE= 22.9); however, there were no significant differences found (F = 2.184, p = 0.119) (Figure 4b).

We also found that the stress indicators (H/L rates) of 81 individual Semipalmated Sandpiper varied significantly (KW = 34.199, p < 0.001) during the winter cycle (Figure 4c), and there was an increase in stress indicators. The lowest average H/L rates were found in October (± SE= 3.6), and the highest were found in April (± SE= 13.6) (Figure 4c). Furthermore, differences in H/L rates were found between October and January (p < 0.001) and October and April (p < 0.001). However, there were no differences between January and April (p = 0.127) (Figure 4c).

Table 1. Seasonal variation in the count of macroinvertebrates during the winter (October, January and April) of *Calidris pusilla* on the Restinga Island, northeast of South America.

Surface availability (m²)	**October**	**January**	**April**	**KW-F**	**P**
Seasonal					
Gastropod	307.6	198.6	145.3	F- 9.609	0.0002
Bivalve	134.3	80.9	51.9	Kw- 12.229	0.002
Polychaete	2.3	1.7	1.6	F- 0.385	0.682
Crustacean	0.2	0.3	0.2	F- 0.135	0.874

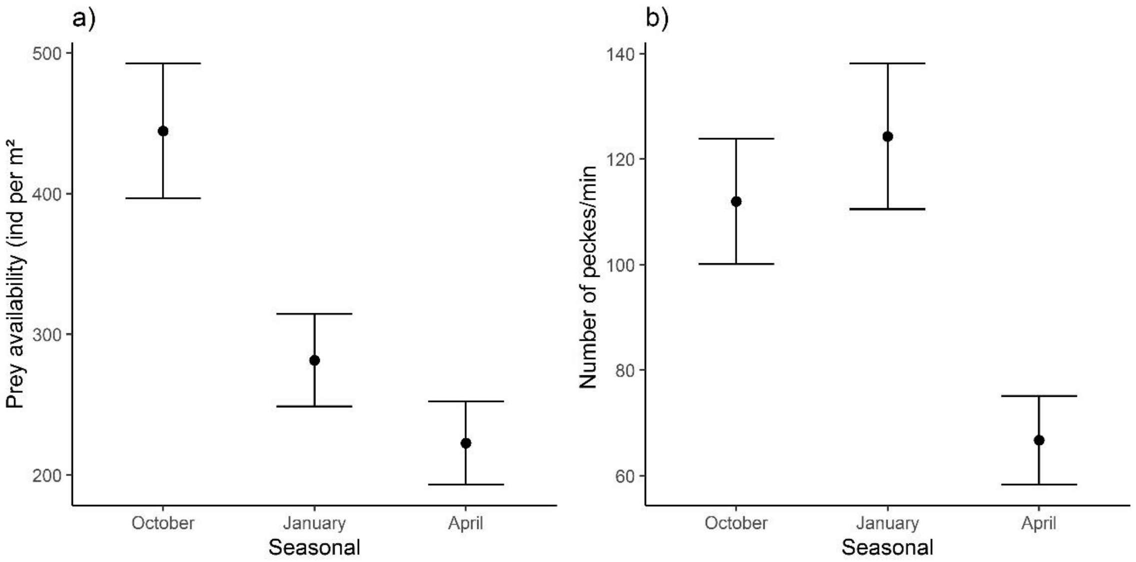

Figure 2. Variation of prey availability (ind. per m²) and variation in the number of pecks/min (October, January, April) of *Calidris pusilla* on the Restinga Island, northeast South America.

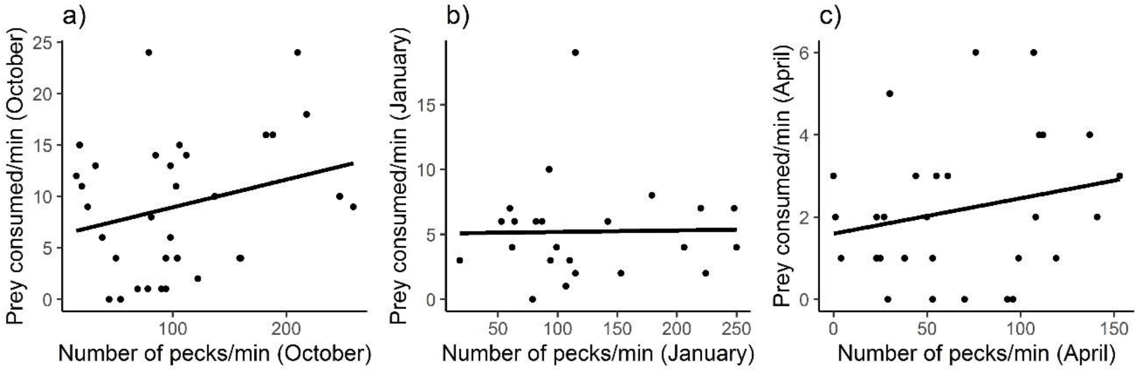

Figure 3. Linear regressions between prey consumed/min and the number of pecks/min during the winter (October, January and April) of *Calidris pusilla* on Restinga Islanda, northeast South America.

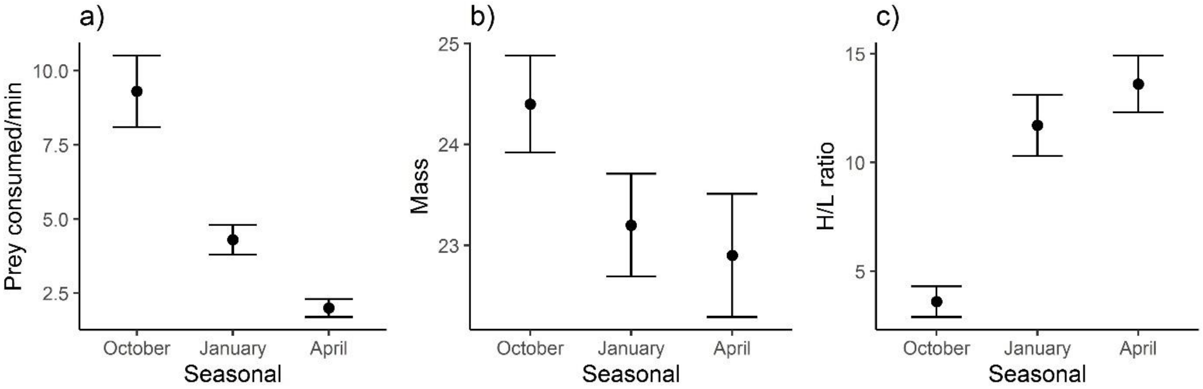

Figure 4. Variation in the prey consumed/min, mass and, H/L ratio of *Calidris pusilla* during its winter (October, January and April) on Restinga Island, northeast of South America.

Discussion

We investigated how Semipalmated Sandpiper adjust the time invested in foraging (pecks/min) and physiological stress indicators (H/L rates) when there is variation of food resources during the winter period. Semipalmated Sandpiper showed a variation in foraging investment throughout the winter months in South America, with an increase in pecks/min up to January and a decrease in April. The reduction of prey availability (ind. per m²), prey consumed/min and mass, as well as the increase in stress indicators (ratios H/L) suggest that the Semipalmated Sandpiper suffered from unpredictable resources and periods of stress during the winter. Therefore, nutrition and physiological stress in Semipalmated Sandpiper was influenced by a lack of environmental food availability, and foraging investment was not able to adjust the consumption to avoid that.

At a particular period of the year, some birds may increase their investment in food consumption to escape the pressures caused by unpredictable food shortages (Gwinner et al. 1985; Bairlein 1988; Landys-Ciannelli et al. 2002; Lendvai et al. 2004). Semipalmated Sandpiper increased the proportion of time spent pecking, with a new behavioural style of foraging, in order to adapt to the decrease in *Corophium volutator* during intertidal mudflats in the Bay of Fundy (MacDonald et al. 2012). In an arid region of the US, *Calidris mauri* showed diversity in the foraging method between two seasons (autumn and spring) due to different proportions of invertebrates (Davis et al. 2001). *Calidris alpine* increased its probe foraging strategy concerning surface pecks due to a decrease in the availability of resources during the winter (Martins et al. 2013).

We recorded similar foraging strategy variations by Semipalmated Sandpiper in the middle of winter; however, this variation did not promote a nutritional or physiological stress adjustment. During the middle of winter in Brazil, the H/L ratio in Semipalmated Sandpiper was associated with the presence of molting and the nutritional costs that molting requires (Silva et al. 2021). Therefore, these foraging strategy variations can also contribute to this energetic demand.

Several groups of animals decrease their foraging behaviours caused by food scarcity due to their perspective of needing to reduce energy expenditure (Westerterp 1977; Dunlap 1995; Hervant et al. 1997; MacCue 2010). This behaviour was seen in individual Semipalmated Sandpiper at the end of the winter period. Thus, periods of food reduction, as seen here, can cause birds to eat less food than they need, thereby increasing their response to nutritional stresses (MacCue 2010), and unpredictable eating conditions can cause changes in birds' stress indicators (Krans et al. 2001; MacCue 2010). For example, the Great Tit (*Parus major*) experienced significant increases in H/L rates during food deprivation in an intense winter period in Latvia (Krans et al. 2011). Additionally, individuals from the Eurasian Treecreeper (*Certhia familiaris*) experienced high H/L rates due to a scarcity and the low quality of food, with increased forest fragmentation (Suorsa et al. 2004). Similar relationships to these were cited for Blue tits (*Cyanistes caeruleus*) and Common Kestrels (*Falco tinnunculus*) (Muller et al. 2011; Banbura et al. 2013). Also, the risk of starvation for birds using areas with unfavourable feeding conditions may increase due to intraspecific and interspecific competitions (Ekman 1987; Krams 1998; Krams et al. 2001). These agonistic behaviours can lead to the uncertainty of survival, which can cause stress levels to increase (Ekman 1987). In general, our results contribute to the hypothesis that combinations of low energy reserves due to food shortages can alter the H/L rates in migratory birds, as seen in Semipalmated Sandpiper at the end of winter. Therefore, these environmental conditions and stress responses can be associated with environmental and intrinsic stimulators for return migration to breeding areas.

In conclusion, our study indicates that Semipalmated Sandpiper experience progressive periods of food shortages and an increase in stress indicators during the winter period in South America. These results help us understand the impact that food variation can cause the migratory cycle of Charadriiformes, as these birds need adequate habitats to supply physiological demand.

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References

Amaral, A.C.Z., and Rizoo, A. P. (2006). Manual for the identification of marine invertebrates in the southeast-south region. *Edi. Da Univer. de São Paulo* **3**, 288.

Bairlein, F. (1988). How do migratory songbirds cross the Sahara? - *Trends in Ecology & Evolution*. **3**, 191–194.

Bańbura, J., Skwarska, J., Bańbura, M., Glądalski, M., Hołysz, M., Kaliński, A. et al. (2013). Spatial and temporal variation in heterophil-to-lymphocyte ratios of nestling passerine birds: comparison of blue tits and great tits *PLoS ONE* **8**,e74226.

Cardoso, T. A., and Zeppelini, D. (2011). Migratory shorebirds during boreal summer and southward migration on the coast of Paraíba, Brazil. *Waterbirds* **34**, 369-375.

Cestari, C. (2015). Coexistence between Nearctic-Neotropical migratory shorebirds and humans on urban beaches of the Southern Hemisphere: a current conservation challenge in developing countries. *Urban Ecosyst*. **18**, 285–291.

Davis, C. A., and Smith, L. M. (2001). Foraging strategies and niche dynamics of coexisting shorebirds at stopover sites in the southern Great Plains. *The Auk* **118**, 484–495.

Dunlap, K. D. (1995). Hormonal and behavioral responses to food and water deprivation in a lizard (*Sceloporus occidentalis*): implications for assessing stress in a natural population. *J. Herpetol.* **29**, 345–351.

Ekman, J. (1987). Exposure and time use in willow tit flocks- the cost of subordination. *Anim. Behav*. **35**, 445–452.

Fedrizzi, C. E., Carlos, C. J., and Campos, A. A. (2016). Annual patterns of abundance of Nearctic shorebirds and their prey at two estuarine sites in Ceará, NE Brazil, 2008–2009. *Wader Study* **123**, 122-135.

Gwinner, E., Biebach, H. and Van-Kries, I. (1985). Food availability affects migratory restlessness in caged garden warblers (*Sylvia borin*). *Naturwissenschaften* **72**, 51–52.

Hicklin, P.W., and Smith, P. C. (1979). The diets of five species of migrant shorebirds in the Bay of Fundy. *Proc. N.S. Inst. Sci*. **29**, 483–488.

Hicklin, P.W., and Smith, P.C. (1984). Selection of foraging sites and invertebrate prey by migrant Semipalmated Sandpiper s, (*Calidris pusilla*), Minas Basin, Bay of Fund. - *Canadian Journal of Zoology* **62**, 2201-2210.

Hervant, F., Mathieu, J., Barre, H., Simon, K., and Pinon, C. (1997). Comparative study on the behavioral, ventilatory, and respiratory responses of hypogean and epigean crustaceans to long-term starvation and subsequent refeeding. *Comp. Biochem. Physiol.* **118**,1277–1283.

Johnstone, P., Richard D. R., and Alan L. (2012). Interpreting indices of physiological stress in free-living vertebrates. *Journal of Comparative Physiology* **182**, 861-879.

Krams, I. (1998). Rank-related fattening strategies of willow tit *Parus montanus* and crested tit *P. cristatus* mixed flock members. *Ornis. Fennica* **75**, 19–26.

Krams, I. A., Krams, T., and Cernihovics, J. (2001). Selection of foraging sites in mixed willow and crested tit flocks: rank-dependent survival strategies. *Ornis Fenn* **78**, 1–11.

Krams, I., Cīrule, D., Krama, T., and Vrublevska, J. (2011). Extremely low ambient temperature affects haematological parameters and body condition in wintering Great Tits (*Parus major*). *J. Ornithol* **152**, 889–895.

Landys-Ciannelli, M. M., Ramenofsky, M., Piersma, T., Jukema, J., Wingfield, J. C., Lendvai, A. Z., et al. (2004). The effect of energy resources on social foraging: hungry sparrows scrounge more. *Proc. R. Soc.* **271**, 2467–2472.

MacDonald, E. C., Ginn, M. G., and Hamilton, D. J. (2012). Variability in foraging behaviour and implications for diet breadth among Semipalmated Sandpiper s staging in the upper Bay of Fundy. *Condor* **114**, 135-144.

Martins, R. C., Catry, T., Santos, C. D., Palmeirim, J. M., and Granadeiro, J.P. (2013). Seasonal Variations in the Diet and Foraging Behaviour of Dunlins *Calidris alpinain* a South European Estuary: Improved Feeding Conditions for Northward Migrants. *PLOS ONE* **8**, e81174.

McCue, M. D. (2010). Starvation physiology: reviewing the different strategies animals use to survive a common challenge. *Compar. Biochem. and Physi. Part A: Molecular & Integrative Physiology.* **156**, 1-18.

Morrison, R.I.G., ROSS, R.K., ANDL, J., and NILES. (2004). Declines in wintering populations of Red Knots in southern South America. *The Condor* **106**, 60–70.

Morrison, R. I. G., Mizrahi, D. S., Ross, R. K., Ottema, O. H., Depracontal, N., and Narine, A. (2012). Dramatic Declines of Semipalmated Sandpiper s on their Major Wintering Areas in the Guianas, Northern South America. *Waterbirds* **35**, 120-134.

Müller, C., Jenni-Eiermann, S., and Jenni, L. (2011). Heterophils/lymphocytes-ratio and circulating corticosterone do not indicate the same stress imposed on *Eurasian kestrel* nestlings. *Funct Ecol* **25**, 566–576.

Owen, J.C., and Moore, F.R. (2006). Seasonal differences in the immunological condition of three species of thrush. *Condor* **108**, 389-398

Piersma, T., Goeij, P., and Tulp, I. (1993). An evaluation of intertidal feeding habitats from a shorebird perspective: Towards relevant comparisons between temperate and tropical mudflats. *Netherl. Journal of Sea Research* **31**, 503–512.

Quinn, J. T., and Hamilton, D.J. (2012). Variation in diet of Semipalmated Sandpiper s (*Calidris pusilla*) during stopover in the upper Bay of Fundy, Canada. *Canadian Journal of Zoology* **90**, e1181.

Rodrigues, R. C., Araujo, H. F. P., Guerra, R. R., Durigon, E. L., Mizrahi, D. S., and Azevedo-Junior, S. M. (2016). Temporal variation in the mass and plumage of four Charadriiformes species on the North-Eastern coast of Brazil. *Emu-Austral Ornithology*, **116**, 461-466

Santos, C.D., Palmeirim, J.M., and Granadeiro, J.P. (2010). Choosing the best foraging microhabitats: individual skills constrain the choices of dunlins *Calidris alpine*. *Journal of Avian Biology* **41**, 18–24.

Silva, L.S.A., Rafael, R.M., Rodrigues, G.G., and de Araujo, H.P (2021). Heterophile/lymphocyte profiles are associated with mass increase and moulting in the Semipalmated Sandpiper *Calidris pusilla* at wintering sites in NE South America. *Acta Ornithol*, **56**, 127–132.

Schaub, M., Jenni, L., and Bairlein, F. (2008). Fuel stores, fuel accumulation, and the decision to depart from a migration stopover site. Behavioral Ecology **19**, 657–666.

Skagen, S. K., and Oman, H. D. (1996). Dietary flexibility of shorebirds in the western hemisphere. *Can. Field-Nat.* **110**, 419-444.

Soursa, P., Helle, H., Koivunen, V., Huhta, E., and Nikula, A. (2004). Effects of forest patch size on physiological stress and immunocompetence in an area-sensitive passerine, the Eurasian treecreeper (*Certhia familiaris*): an experiment. *Proc. R. Soc. Lond. B.* **271**, 435–440.

Westerterp, K. (1977). How rats economize-energy loss in starvation. *Physiol. Zool.* **50**,331–362.

CONSIDERAÇÕES FINAIS

Em conclusão, os resultados dessa pesquisa estão de acordo com a hipótese geral inicialmente proposta. Encontramos que as fases que compreendem o ciclo migratório de *Calidris pusilla* apresentam variações fisiológicas em relação a sua demanda energética, além disso, *Calidris pusilla* variou o investimento de forrageio para atender as suas demandas fisiológicas, assim como para se prevenir contra imprevisibilidade de presas. Os resultados encontrados para esse estudo podem ser importantes para futuras pesquisas que abranjam esse contexto e, busquem medidas de conservação para áreas de forrageios de aves limícolas neárticas. Além disso, eles podem contribuir nos esforços de conservação que buscam entender o rápido declínio enfrentado por *Calidris pusilla* durante o seu período de invernada na América do Sul.