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MARINHA POR AVES SOBRE TEIAS TRÓFICAS
INSULARES

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RESUMO

Aves marinhas transportam nutrientes e energia entre áreas de forrageamento no mar e áreas de reprodução em terra. O transporte de matéria marinha para o ambiente terrestre proporciona a fertilização do solo e o fornecimento de energia para diferentes níveis tróficos, capazes de gerar dependência entre teias tróficas marinhas e terrestres. O Arquipélago de Fernando de Noronha é a mais importante área para a reprodução de aves marinhas no Brasil, porém abriga espécies de vertebrados exóticos invasores que predam ovos e filhotes de aves, algumas das quais ameaçadas de extinção. Por serem potenciais transportadoras de nutriente marinho as aves podem sustentar teias tróficas insulares e, assim, essas interações precisam ser melhor compreendidas. Análises de isótopos estáveis (AIE) de carbono e nitrogênio indicaram a presença e o fluxo de matéria marinha ao longo das cadeias tróficas. A contribuição dos itens alimentares foi determinada através do Índice de Importância Relativa Presa-específica (%PSIRI) e de modelos de mistura isotópica bayesianos. Hipotetizamos que a teia trófica insular é subsidiada por matéria de origem marinha transportada pelas aves marinhas, e que espécies exóticas invasoras utilizam esses nutrientes através do consumo direto e indireto. Ainda, predizemos que o aumento dos valores de $\delta^{15}\text{N}$ no ambiente se dissipe conforme o aumento da distância da fonte, e que espécies exóticas invasoras apresentem $\delta^{13}\text{C}$ de origem marinha em seus tecidos. Em duas transecções terrestres contínuas, com 50 m cada, iniciando no local de deposição (colônia ou área de descanso) foram coletadas amostras de plantas C3 e C4, formigas, aranhas e caranguejos terrestres. Também foram coletadas amostras de sangue, fezes e conteúdo estomacal de ratos (*Rattus rattus*), gatos (*Felis catus*) e teiús (*Salvator merianae*), para análise da dieta, e de fontes alimentares potenciais destes consumidores. A AIE evidenciou o uso de itens alimentares de origem marinha por todos os consumidores terrestres, incluindo vertebrados exóticos invasores e invertebrados terrestres. Os valores de $\delta^{15}\text{N}$ em plantas decresceram conforme o aumento da distância da fonte, e o enriquecimento isotópico foi detectado até 100 m de distância da colônia, porém com redução significativa. O lagarto endêmico (*Trachylepis atlantica*) teve alta contribuição na alimentação de todas as espécies exóticas. Aves contribuíram na dieta de gatos e ratos. No geral, demonstramos a importância dos nutrientes de origem marinha carregado por aves marinhas, para as teias tróficas insulares, incluindo vertebrados

exóticos invasores. Adicionalmente, o impacto de espécies exóticas sobre espécies endêmicas e ameaçadas foi demonstrado. Os resultados reforçam a necessidade de adoção de medidas de conservação de espécies ameaçadas, através do manejo de vertebrados invasores nas ilhas. Tais medidas podem contribuir diretamente para o aumento populacional das espécies em risco de extinção, e indiretamente promover o aumento do aporte de nutrientes nas ilhas, restabelecendo o funcionamento do ecossistema em níveis mais próximos daqueles que ocorriam antes da chegada humana, quando as populações de aves eram maiores e com distribuição mais ampla no arquipélago.

Palavras-chave: Ecologia trófica; Gatos ferais; Ilhas oceânicas; Material alóctone Transporte Lateral.

ABSTRACT

Nutrients and energy are carried by seabirds from sea, feeding grounds to breeding areas on land. In islands, the transported marine materials fertilize soil and provide energy for several trophic levels, which may result in dependency between marine and terrestrial trophic webs. Fernando de Noronha Archipelago is the most important breeding ground for seabirds in Brazil, but the introduction of invasive exotic vertebrates that prey upon eggs and chicks is a threat to seabirds. Seabirds are potential carriers of marine nutrients that sustain terrestrial trophic webs, the main reason for these interactions to be better understood. In the present work, stable isotope analysis (SIA) was used to identify the marine matter presence and its flow through trophic chains. Contributions of food items were determined for Prey-Specific Index of Relative Importance (%PSIRI) and Bayesian isotopic mixing models. Our hypothesis is that the terrestrial trophic web is maintained by marine matter carried by seabirds, and invasive species use this nutrient through direct and indirect ways. We predict that $\delta^{15}\text{N}$ values are reduced over long distances from sources, and exotic species have marine $\delta^{13}\text{C}$ signatures in their tissues. C3 and C4 plants, ants, spiders and land crabs were sampled over two continuous 50 m-long transects. Blood, scats and stomach contents of black rats (*Rattus rattus*), feral cats (*Felis catus*) and tegu lizards (*Salvator merianae*) were collected for diet analysis, in addition to potential food items. SIA provided evidences of high contribution of marine sources to all terrestrial consumers, including exotic vertebrates and invertebrates. Values of $\delta^{15}\text{N}$ decreased in plant samples according to increased distance from sources, and the isotopic enrichment was detected up to 100 m from the source, but with significant reduction. Diet analysis shows high contribution of the endemic skink (*Trachylepis atlantica*) as food resource for all exotic species, while birds had larger contribution on feral cat and rat diets. This study highlights the importance of nutrients carried by seabirds for terrestrial trophic webs, and the impact of exotic species upon threatened species. It also demonstrates the need for adoption of conservation measures focused on threatened species, through the management of vertebrate invasive species on islands. Such measures could contribute directly for the increase in population size of seabird species under threat, as well as indirectly, by promoting an increase in nutrient inputs to terrestrial ecosystems, and thus establish ecosystems functions at levels more closely to those before

human arrival, when seabird populations were larger and colonies more widely distributed on the Archipelago.

Keywords: Allochthonous matter; Feral cats; Lateral transport; Oceanic islands; Trophic ecology.

INTRODUÇÃO

Organismos capazes de se mover entre fronteiras ecossistêmicas, como as aves marinhas que utilizam o ambiente marinho e terrestre, podem transportar nutrientes e energia entre ecossistemas distantes ou adjacentes (Polis et al. 1997, Korobushkin 2014), como entre áreas de forrageamento no mar e áreas reprodutivas em terra. Os nutrientes introduzidos no sistema terrestre através do guano, regurgitados, ovos ou carcaças (Anderson & Polis 1998), fertilizam o ambiente e fornecem energia para os diferentes níveis tróficos (McLoughlin et al. 2016). Ambientes insulares recebem com frequência o aporte de nutriente marinho (Polis et al. 1997) e este subsídio pode atuar como principal fonte de energia (Polis & Hurd 1995), modificar a dinâmica entre consumidores e recursos (Bauer & Hoyer 2014) e conectar teias tróficas marinhas e terrestres (McLoughlin et al. 2016).

As colônias de aves marinhas fornecem energia de forma direta (Polis & Hurd 1995) aos predadores de ovos, filhotes e àqueles que se alimentam de carcaças (Sanchez-Piñero & Polis 2000); de forma indireta enriquecem a cadeia com resíduos marinhos no solo que serão utilizados pelos produtores e consumidores de detritos (Caut et al. 2012). A intensidade do transporte lateral de nutrientes varia de acordo com a produtividade dos ambientes adjacentes, em geral ocorrendo no sentido do ambiente mais produtivo para o menos produtivo (Paetzold et al. 2008). Portanto, a relação entre os sistemas pode gerar dependência das espécies insulares terrestres ao aporte resultante da produtividade aquática (Stapp et al. 1999). Aves marinhas são capazes de viajar longas distâncias em busca de alimento, por exemplo, o albatroz-errante (*Diomedea exulans*) desloca-se para áreas a mais de 3000 km de distância da colônia, localizada nas ilhas Geórgia do Sul, e retorna para alimentar o filhote (Prince et al. 1992). Aves da família Sulidae realizam viagens de forrageio até 100 km de distância de seus ninhos e utilizam áreas de até 300 km² para se alimentar (Young et al. 2015). Dessa forma, são capazes de transportar elementos-traço para o solo próximo a suas colônias e enriquecer o ambiente com nitrogênio em mais de 100 vezes, através da deposição de guano (Mulder et al. 2011). Por isso, é possível encontrar predadores terrestres enriquecidos em ¹⁵N derivado de matéria marinha, em locais próximos à colônia, mas também até 200 m de distância desta fonte (Hobson et al. 1999, Stapp & Polis 2003, Korobushkin 2014). Quatro ilhas localizadas

em diferentes latitudes e longitudes foram utilizadas para exemplificar o padrão geral de dissipação de nutrientes, e em todas elas foram encontrados valores de $\delta^{15}\text{N}$ muito superiores em áreas próximas às colônias do que em áreas distantes da fonte (Caut et al. 2012). Além disso, análises de elementos-traço em ilhas com e sem colônias de aves marinhas revelam que ilhas com colônias apresentam composição química única com elevadas concentrações de elementos como potássio, cálcio e zinco (Mallory et al. 2015), se comparadas às ilhas sem colônias. Por isso aves marinhas são capazes de influenciar a qualidade do habitat de ambientes costeiros e, conseqüentemente, os organismos associados a estes locais.

O fluxo de carbono e nitrogênio dentro de teias alimentares dissipa-se em rotas invisíveis. No entanto, a análise de isótopos estáveis (AIE) permite identificar e interpretar essas vias (Rounick & Winterbourn 1986). Os valores de $\delta^{13}\text{C}$ e $\delta^{15}\text{N}$ são, respectivamente, 7‰ e 1‰ mais baixos na atmosfera do que no oceano (Peterson & Fry 1987). No ambiente marinho o valor médio de $\delta^{13}\text{C}$ nas algas é de -21‰ (Peterson & Fry 1987, Fry 2006), enquanto no ambiente terrestre, plantas C3 têm valores de $\delta^{13}\text{C}$ em torno de -28‰, e plantas C4 têm valores entre -14‰ e -10‰. Além disso, valores de $\delta^{15}\text{N}$ representam o nível trófico com eficiência, devido ao acúmulo de ^{15}N nos tecidos, que resulta em um aumento de 3‰ a 4‰ em relação ao nível trófico anterior (Peterson & Fry 1987). Por isso, a análise da composição isotópica é pertinente para avaliar a presença de matéria marinha na teia trófica terrestre.

Isótopos estáveis são átomos de um mesmo elemento químico, porém com diferentes números de nêutrons presentes em seu núcleo, o que reflete em massas atômicas distintas (Peterson & Fry 1987). Isótopos estáveis, assim como os demais elementos, são utilizados em diferentes reações químicas dos organismos, entretanto, devido à diferença em suas massas, átomos mais leves tendem a reagir mais rapidamente devido à alta energia e baixa estabilidade, por isso são utilizados preferencialmente em reações metabólicas como a excreção (Peterson & Fry 1987). Já os átomos com maior massa possuem menor energia reativa e, portanto, realizam ligações mais estáveis. Por isso, são retidos predominantemente na formação dos tecidos dos organismos (Peterson & Fry 1987).

AIEs demonstram efetivamente o alimento assimilado pelo tecido do consumidor durante determinado período, apesar de não avaliar a dieta com alta resolução taxonômica

(Rounick & Winterbourn 1986). Por isso a análise de dieta convencional, realizada através de análises de conteúdos estomacais ou amostras de fezes (Bonnaud et al. 2007), pode ser utilizada como uma ferramenta complementar à AIE. Através da análise de dieta convencional, é possível identificar de forma precisa as espécies ingeridas (Sih & Christensen 2001), visto que a técnica está baseada em identificação e quantificação do conteúdo, seja ele fezes, regurgitados, ou conteúdo estomacal. A principal limitação desta técnica está relacionada à digestibilidade de partes moles, muitas vezes necessárias para a identificação de espécies e ao fato de informar somente sobre um período curto de alimentação do consumidor (Barret et al. 2007). Contudo, o uso simultâneo das técnicas de AIE e de dieta convencional aumenta a resolução taxonômica da cadeia trófica estudada, ao mesmo tempo em que avalia itens alimentares importantes na síntese de tecidos dos consumidores ao longo de períodos mais longos e, assim, possibilita a obtenção de resultados mais completos.

A invasão de habitats insulares por espécies exóticas é um problema global (Funk 2015) que tem levado diversas espécies à extinção ou a um elevado risco de extinção. Entre as espécies recentemente extintas no planeta, 61% habitavam ilhas, e 37% das espécies criticamente ameaçadas que constam na Lista Vermelha global (IUCN 2010) são espécies insulares (Tershy et al. 2015). A presença de espécies exóticas pode afetar negativamente a estrutura populacional de espécies nativas, reduzir a amplitude do nicho realizado e afetar diretamente a estrutura e o funcionamento das comunidades biológicas e dos processos ecológicos (Lockwood et al. 2007). A introdução de camundongos (*Mus musculus*) nas ilhas Gough, Oceano Atlântico sul, por exemplo, tem levado à alta mortalidade de filhotes de albatroz-de-tristão (*Diomedea dabbenena*) o que, juntamente com a captura incidental na pesca, refletiu em uma projeção de extinção da espécie em aproximadamente 30 anos (Wanless et al. 2009). Em outro exemplo, porcos ferais introduzidos na Austrália têm afetado a estrutura e a composição das comunidades vegetais, levando à diminuição de populações com hábito alimentar seletivo, alterando a ciclagem de nutrientes e dispersando doenças (Bengsen et al. 2014).

A biota de ilhas é fortemente afetada pela ação antrópica, por serem ambientes complexos e com altas taxas de endemismo (Kier et al. 2009, Wetzel et al. 2013). Além disso, o aumento populacional humano tem correlação positiva com a introdução de animais exóticos (Glen et al. 2013). O Arquipélago de Fernando de Noronha diferencia-

se das demais ilhas oceânicas brasileiras justamente pelo seu histórico de ocupação humana (Serafini et al. 2010). O arquipélago recebe cerca de 2000 turistas por mês e possui população permanente de cerca de 3000 habitantes (IBGE 2017). Fernando de Noronha é a mais importante área para a reprodução de aves marinhas no Brasil, pela riqueza (11 espécies) e abundância (Mancini et al. 2016). Dentre as espécies de aves marinhas que reproduzem no arquipélago, quatro estão ameaçadas de extinção no Brasil (MMA 2014). O atobá-de-pé-vermelho (*Sula sula*) e os rabos-de-juncos (*Phaethon lepturus* e *Phaethon aethereus*) são classificados como “Em perigo”, enquanto a pardela-de-asa-larga (*Puffinus lherminieri*) é listado como “Criticamente Ameaçada” devido ao tamanho reduzido da sua população (MMA 2014). Três espécies de aves terrestres nativas também habitam o arquipélago, a pomba arribaçã (*Zenaida auriculata*), e os Passeriformes juruviara-de-noronha (*Vireo gracilirostris*) e cucuruta-de-noronha (*Elaenia ridleyana*), cujas duas últimas são endêmicas e costumam ser avistadas em áreas mais arborizadas (Schulz-Neto 2004). Pouco se sabe sobre a diversidade de invertebrados terrestres no arquipélago, entretanto há registros de 3 espécies endêmicas de gastrópodes (Gomes et al. 2006), 2 espécies de escorpiões e 44 espécies de aranhas (Freitas 2007). O invertebrado que recebe maior atenção, no entanto, é o caranguejo-amarelo (*Johngarthia lagostoma*), endêmico das ilhas oceânicas do Oceano Atlântico sul (Hartnoll et al. 2006) e que apesar de ameaçado na lista brasileira (Em perigo), se alimenta oportunisticamente de ovos e aves jovens (López-Victoria & Werding 2008). No arquipélago também habitam espécies exóticas invasoras, como os roedores *Rattus rattus* e *R. norvegicus*, o teiú *Salvator* (= *Tupinambis*) *merianae* e o gato-doméstico *Felis catus*, que destacam-se por predarem ovos, aves jovens, e o lagarto endêmica *Trachylepis atlantica*, conhecida popularmente como mabuia (Caut et al. 2008a, Cepan 2009).

A descaracterização do ambiente no arquipélago é consequência do histórico de ocupação humana, das pressões antrópicas que foram surgindo e da introdução de espécies invasoras. Após a ocupação portuguesa durante o século XVIII, o arquipélago funcionou como presídio e teve praticamente toda a sua vegetação arbórea retirada, para evitar que os presos construíssem jangadas (Teixeira et al. 2003). Além disso, espécies de aves nativas foram alvo de caça para consumo e produção de souvenirs, até a implantação do Parque Nacional Marinho, em 1988 (Nacinovic & Teixeira 1989). Animais domésticos como cabras, porcos, galinhas e vacas também foram introduzidos

na ilha de Fernando de Noronha (principal) para servir como alimento, mas principalmente as espécies pastadoras foram responsáveis pelo pisoteio e destruição da vegetação remanescente, provocando erosão e afetando a fauna associada (Serafini et al. 2010). Espécies domésticas como cães e gatos também foram trazidas para o arquipélago e assim como os ratos, predam a fauna nativa (Oren 1984, Bellini & Sales 1992) e disseminam zoonoses, como a toxoplasmose, problemas que persistem atualmente (Magalhães et al. 2016).

Supostamente os ratos foram introduzidos de forma acidental, associados à colonização humana do arquipélago, chegando através dos navios europeus (Leão et al. 2011). São onívoros, porém causam grandes impactos às aves nativas e residentes ao alimentar-se de ovos e aves jovens (Lowe et al. 2000, Stapp 2002). O lagarto teiú foi introduzido em Noronha supostamente com o objetivo de realizar o controle biológico dos ratos (Guimarães 2015), porém não encontrou predadores naturais ou melhores competidores e passou a alimentar-se do lagarto mabuia (Leão et al. 2011) e de ovos de aves nativas, ampliando sua população (Schulz-Neto 2004, Cepan 2009). Gatos domésticos são predadores oportunistas (Dauphiné & Cooper 2009) e vetores de doenças como a toxoplasmose, a qual apresenta prevalência de uma cepa única em mais de 70% da população de gatos do arquipélago (Magalhães et al. 2016). Gatos estão presentes em 34 Unidades de Conservação no Brasil (Sampaio & Schmidt 2013), e foram responsáveis pela extinção de pelo menos oito espécies de aves em ilhas no mundo (Lowe et al. 2000).

As baixas taxas de sucesso reprodutivo de aves marinhas se devem, entre outros fatores, à introdução de espécies exóticas (Russell & Le Corre 2009) e à consequente predação de ovos e filhotes (Yang et al. 2014). Essa atividade é realizada principalmente por ratos, causa da grande mortalidade de filhotes de aves tropicais durante o período reprodutivo (Leal et al. 2016). Aves marinhas que nidificam nas ilhas Shiant, na Escócia, têm declinado desde a introdução acidental de ratos, em 1900, devido ao uso de aves e invertebrados marinhos como principal fonte alimentar (Stapp 2002). Populações de aves terrestres também sofrem com a predação e competição com espécies exóticas introduzidas. Nas ilhas Falkland, Malvinas, por exemplo, duas espécies de Passeriformes (*Troglodytes cobbi* e *Cinclodes antarcticus*) foram extintas localmente e deslocadas para outras ilhas do arquipélago devido à interação com ratos introduzidos (Tabak et al. 2016). Por outro lado, as aves marinhas são potenciais transportadoras de nutrientes, capazes de

sustentar direta e indiretamente teias tróficas em ilhas. Atuam como importantes elos entre os sistemas marinho e terrestre, mas são afetadas pela ação de espécies exóticas invasoras em Fernando de Noronha, o que pode limitar o tamanho das populações e a distribuição das colônias. Por isso, estas conexões precisam ser melhor compreendidas.

O objetivo desse trabalho é, portanto, determinar o padrão de dissipação de matéria marinha incorporados por organismos terrestres e os efeitos do transporte de nutrientes realizado pelas aves marinhas na teia trófica insular. Para isso, avaliamos no primeiro capítulo: (i) a existência de transporte de matéria marinha para o ambiente terrestre mediado por aves marinhas; (ii) o padrão de dispersão espacial (horizontal) dos subsídios marinhos ao longo do ambiente insular e entre as estações do ano (temporal) (iii) a contribuição de matéria marinha na dieta de consumidores terrestres em diferentes níveis tróficos (dissipação vertical). No segundo capítulo verificamos a utilização de espécies nativas e endêmicas como recurso alimentar na dieta de gatos, ratos e teiús. Primeiramente, hipotetizamos que produtores primários apresentariam aumento dos valores de $\delta^{15}\text{N}$ (assinatura marinha) derivado de matéria marinha transportada por aves para a ilha. Além disso, valores de $\delta^{15}\text{N}$ seriam reduzidos conforme o distanciamento da fonte (Fig. 1). Segundo, hipotetizamos que consumidores terrestres apresentariam valores de $\delta^{13}\text{C}$ de origem marinha, e que espécies nativas e endêmicas contribuem significativamente para a dieta de gatos, ratos e teiús (Fig. 2).



Fig. 1 Predição do padrão de dissipação horizontal dos nutrientes de origem marinha conforme o distanciamento da fonte, com base em análises de isótopos estáveis de nitrogênio em tecidos de produtores.

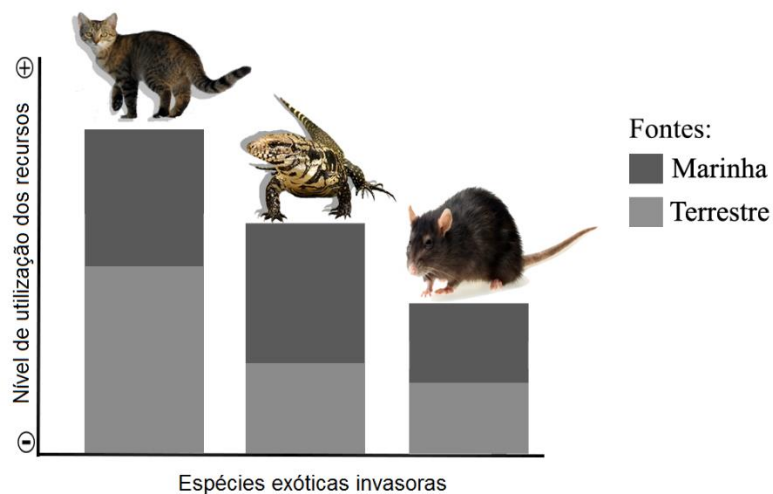


Fig. 2 Predição do nível de contribuição de matéria marinha e terrestre para três espécies de vertebrados exóticos invasores no arquipélago de Fernando de Noronha. As proporções são baseadas no esperado com relação ao hábito alimentar de cada espécie, entretanto gatos, ratos e teiús apresentarão contribuição marinha em sua dieta.

MATERIAL E MÉTODOS

Área de estudo

A amostragem foi realizada nas ilhas de Fernando de Noronha (principal) e do Meio, no Arquipélago de Fernando de Noronha (3°52'S; 32°26'W), distante 360 km do continente. O Arquipélago é composto por 21 ilhas vulcânicas e tem área de 26 km² (Schulz-Neto 2004). O clima é tropical e caracterizado por duas estações bem definidas, a seca (de agosto a janeiro) e a chuvosa (de fevereiro a julho), com pluviosidade média anual de 1400 mm (Teixeira et al. 2003). Durante as duas estações é possível notar diferenças marcantes na vegetação devido a sua característica decídua (Fig. 3). A cobertura vegetal do arquipélago é composta por 331 espécies de plantas vasculares, entre nativas e exóticas (Alves 2006), sendo possível encontrar espécies arbóreas, arbustivas e herbáceas (Mello & Oliveira 2016).

A ilha principal é a única no arquipélago com população humana residente, e a ilha do Meio pertence ao conjunto de áreas protegidas e não habitadas componentes do Parque Nacional Marinho de Fernando de Noronha (PARNAMAR- FN). O Parque perfaz 11.270 ha do arquipélago, o que corresponde a 70% da ilha principal e todas as ilhas

secundárias. As demais áreas pertencem à Área de Proteção Ambiental e têm o turismo e a ocupação regidos pelo plano de manejo do arquipélago. Caracterizado como santuário de diversas espécies desde 2001, o PARNAMAR de Fernando de Noronha é tombado pela UNESCO como Patrimônio Natural Mundial da Humanidade. Além disso, é considerado como *Important Bird Area* conforme as categorias da BirdLife International (Devenish et al. 2009) e Sítio RAMSAR, categoria dada às áreas que concentram as mais representativas áreas úmidas do planeta.

A ilha do Meio é local de reprodução de aves marinhas visitantes, como o atobá-mascarado (*Sula dactylatra*), o qual forma colônias durante as estações seca e chuvosa. O atobá-de-pé-vermelho pode ser encontrado na ilha durante todo o ano, entretanto os ninhos podem ser notados apenas durante a estação seca (Fig. 4). A ilha abriga ainda, o caranguejo-amarelo, o lagarto endêmico mabuia e o invasor rato-preto (*R. rattus*).



Fig. 3 Modificação da estrutura da vegetação no ninhal de atobá-mascarado (*S. dactylatra*), localizado na ilha do Meio, durante as estações de seca (esquerda) e chuvosa (direita).



Fig. 4 Diferenças nas características da vegetação e uso do habitat por atobás-de-pé-vermelho na ilha do Meio. Durante a estação seca é possível observar a presença de alguns

ninhos (esquerda), enquanto no período de chuva este mesmo local é utilizado apenas como área de descanso e dormitório (direita).

Coleta de dados

As coletas de amostras para o capítulo 1 foram realizadas em outubro de 2016 e abril de 2017, a fim de contemplar as duas estações do ano no Arquipélago. Na ilha do Meio, foi determinada uma transecção de 100 m (Fig. 5), dividida em duas secções de 50 m, que partem de possíveis áreas de aporte de nitrogênio (áreas fonte), realizada conforme adaptação do método utilizado por Korobushkin (2014). As áreas fonte escolhidas foram o ninhal de atobá-mascarado (Dcol), e a área de descanso no interior da ilha (Droost). O Dcol permanece na ilha durante as duas estações do ano, enquanto Droost funciona como dormitório na estação de chuva e como área reprodutiva de atobás-de-pé-vermelho durante a seca, porém com poucos ninhos (Fig. 4). As coletas foram realizadas em pontos localizados a 0, 5, 10, 20 e 50 m de distância das áreas fonte, de forma que o ponto 0 está localizado dentro das áreas fonte e o ponto 50 é intermediário às duas áreas (Fig. 6). Em cada ponto de coleta foram instaladas 3 armadilhas pitfall e amostradas folhas de plantas com ciclo fotossintético C3 e C4. Cada pitfall foi verificada no máximo a cada dois dias no período da manhã e as formigas e aranhas capturadas foram conservadas em álcool 70%. As amostras de plantas foram secas ao sol e armazenadas em sacos zip-lock.

Amostras de músculo de caranguejo-amarelo, além de fígado e músculo de rato-preto foram coletadas para avaliar a dieta e a presença de carbono de origem marinha nos tecidos dos consumidores. Sangue de atobás-mascarados e folhas de plantas C3 foram utilizados como *endpoint* marinho e terrestre, respectivamente. As amostras de músculo de caranguejo foram coletadas de forma não letal, através da remoção de um pereiópodo. Ratos foram capturados com puçá e armadilhas Tomahawk (50 × 21.5 × 20 cm), eutanasiados e tiveram o estômago removido para análise de conteúdo estomacal (CEUAVET-FMVZ-USP número 1713240815). Teiús foram eutanasiados utilizando uma associação de quetamina (2-30 mg/kg) e midazolan (1-2 mg/kg) seguido de perfuração cerebral. Também tiveram o conteúdo estomacal coletado através de necropsia (CEUAVET-FMVZ-USP número 2724150515). Fezes de gatos ferais foram coletadas ao longo de trilhas em áreas não habitadas da ilha principal, durante todo o ano de 2015 (permissão SISBIO 41682-6) e cedidas pela FMVZ-USP. Potenciais fontes alimentares

foram selecionadas com base nos resultados das análises de dieta e dados disponíveis na bibliografia (Stapp 2002, Castro & Galletti 2004, Bonnaud et al. 2007, Dickman 2009, Silva et al. 2014). Foram amostrados frutos de diferentes árvores e músculo das mabuias, através da autotomia da cauda após captura manual. Amostras de tecido animal foram armazenadas em álcool 70%, enquanto frutos e fezes de gato foram armazenados em sacos zip-lock e congelados.

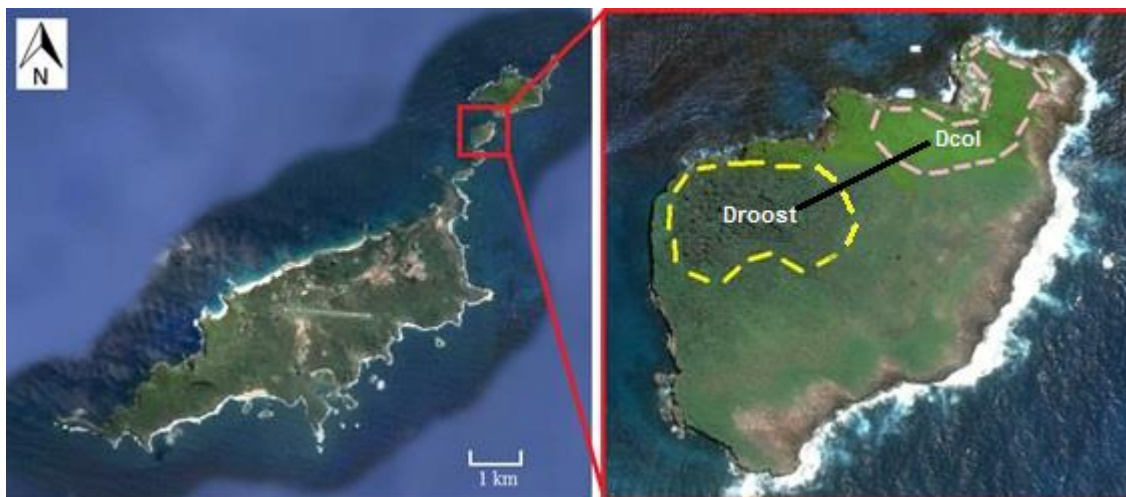


Fig. 5 Área de estudo (ilha do Meio), localizada a nordeste da ilha Principal do arquipélago de Fernando de Noronha. Na ilha do Meio as potenciais áreas de aporte de nitrogênio estão destacadas em amarelo e rosa. A transecção de 100 m (linha em preto) foi definida entre um ninhal de atobás-mascarados (Dcol) e uma área de descanso de atobás-de-pé-vermelho (Droost).

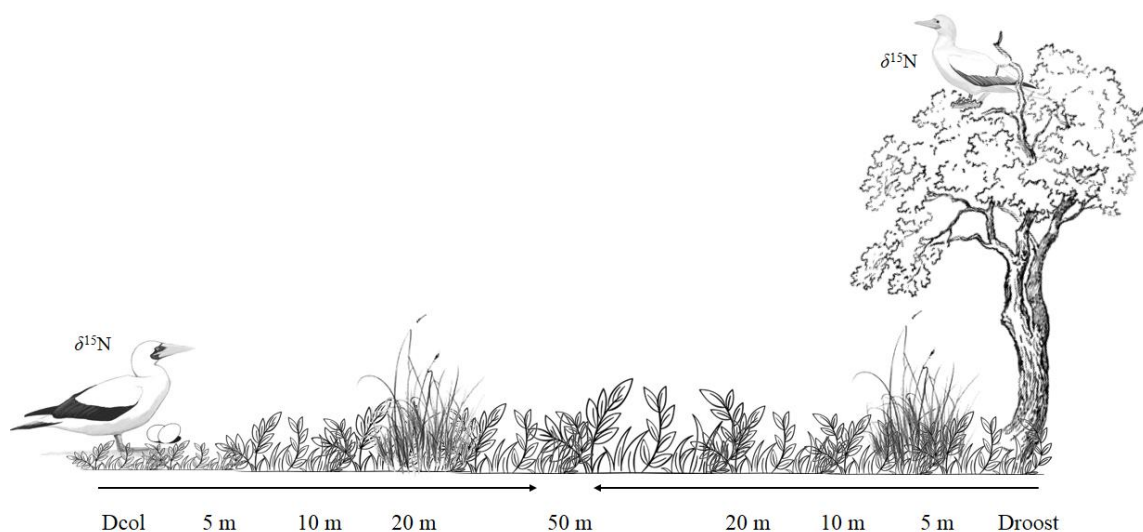


Fig. 6 Transecção amostrada na ilha do Meio no Arquipélago de Fernando de Noronha. A transecção de 100 m foi dividida em duas secções de 50 m cada, com pontos específicos de coleta que vão se distanciando das áreas fonte – Dcol e Droost – até convergirem a 50 m.

Análise de dieta

Conteúdos estomacais de teiús e ratos foram analisados em lupa estereoscópica (10 × 40) e os itens alimentares foram identificados ao menor nível taxonômico possível. Amostras de fezes de gato foram lavadas em água quente corrente sob peneira de 0.5 mm, seguindo a metodologia de Nogales et al. (1988). Todos os itens encontrados foram separados e identificados com auxílio do material de referência do Laboratório de Aves e Tartarugas Marinhas.

Os itens alimentares foram quantificados em número e volume, utilizando itens diagnósticos únicos (e.g. crânio, mandíbula) ou em pares (e.g. patas, asas), e placa de contagem com papel milimetrado. O volume dos itens foi mensurado conforme a metodologia de Hellawell & Abel (1971) em milímetros cúbicos e em seguida transformados em ml, de acordo com a relação $1 \text{ mm}^3 = 0,001 \text{ ml}$.

Para determinar a importância relativa presa-específica, utilizamos o índice %PSIRI (Brown et al. 2011), calculado através da equação:

$$\%PSIRI = \frac{[(\%PN + \%PV) \times \%FO]}{2} \quad (\text{eq. 1})$$

cujas medidas utilizadas foram obtidas a partir da frequência de ocorrência (FO), da abundância ou contribuição numérica (N) e do volume (V). A FO é dada pelo número de amostras que contém o item alimentar selecionado e a frequência de ocorrência relativa (%FO) é a porcentagem deste número em relação ao total de amostras analisadas de cada espécie de consumidor. O valor de N é dado através da contagem do número de presas consumidas, avaliada através do item alimentar que apareceu na amostra (i.e. o número de indivíduos predados ou de frutos consumidos). A média das porcentagens equivalentes ao N de cada amostra, excluindo-se as amostras em que o item alimentar não esteve presente é a proporção numérica (%PN). Da mesma forma, o volume total de um item alimentar (V) é dado pela soma dos valores de volume obtidos em todas as amostras, enquanto a proporção volumétrica relativa (%PV) é dada pela porcentagem deste item em relação ao volume total das amostras em que o item esteve presente.

Análise de isótopos estáveis

O material vegetal foi macerado, pesado (3 mg) e armazenado em cápsulas de estanho. Aranhas e formigas tiveram o corpo todo utilizado devido ao tamanho pequeno. Foram liofilizadas, homogeneizadas, pesadas (1 mg) e encapsuladas. Amostras de músculo tiveram os lipídios extraídos através do aparato de Soxhlet, segundo a metodologia empregada por Mancini & Bugoni (2014). Em seguida, as amostras foram liofilizadas, homogeneizadas, pesadas (1 mg) e armazenadas em cápsulas de estanho. Todas as amostras foram enviadas para análise em Espectrômetro de Massa de Razão Isotópica (IRMS) no Centro de Isótopos Estáveis da *University of New Mexico* (UNM-CSI). O padrão utilizado para análise de carbono e nitrogênio foi o belemnito PeeDee de Vienna (VPDB) e ar atmosférico, respectivamente. O desvio padrão para valores de $\delta^{13}\text{C}$ e $\delta^{15}\text{N}$ foi de 0,08‰ e 0,03‰, respectivamente, baseado em valores padronizados obtidos através dos valores isotópicos de soja, caseína, *wey protein* e atum. A determinação dos valores foi expressa por δ em partes por mil (‰), conforme a equação de Bond & Hobson (2012):

$$\delta^{13}\text{C} \text{ ou } \delta^{15}\text{N}(\text{‰}) = \left(\frac{R_{\text{amostra}}}{R_{\text{padrão}}} - 1 \right) \quad (\text{eq. 2})$$

em que R é a razão isotópica representada por $^{13}\text{C}/^{12}\text{C}$ ou $^{15}\text{N}/^{14}\text{N}$ para $\delta^{13}\text{C}$ ou $\delta^{15}\text{N}$, respectivamente.

Análise de dados

Todas as análises estatísticas foram realizadas no software R (R Core Team 2014). Foram testadas a normalidade e a homocedasticidade dos dados para todas as análises através do teste de Shapiro-Wilk e Levene, respectivamente. No capítulo 1, para testar o efeito da variável estação (seca vs. chuvosa) sobre a variação dos valores de $\delta^{15}\text{N}$ ao longo do transecto, foi utilizado teste t usando a função “lm”. Para testar o efeito da variável distância também sobre a variação dos valores de $\delta^{15}\text{N}$, foi utilizada ANOVA de uma via com função “aov”. Quando significativo, foi utilizado a análise *post-hoc* para verificar entre quais pontos de coleta houve variação de nitrogênio, através do teste de Tukey.

Nos capítulos 1 e 2, para identificar a origem do $\delta^{13}\text{C}$ (i.e. marinho ou terrestre) e a contribuição relativa dos itens alimentares nos tecidos dos consumidores, utilizamos modelos de mistura isotópica bayesianos através do pacote SIAR (Parnell et al. 2010). A seleção das potenciais fontes alimentares das espécies exóticas foi feita com base nos itens encontrados nas análises de conteúdo estomacal, fezes, e em estudos anteriores disponíveis na literatura para gatos, teiús e ratos (Kiefer & Sazima 2002, Bonnaud et al. 2007, Caut et al. 2008a). Para avaliar a contribuição marinha e terrestre na cadeia trófica, utilizamos valores isotópicos do sangue de atobá-mascarado, coletados em Fernando de Noronha e disponíveis no banco de dados do Laboratório, e folhas de plantas C_3 como *endpoints* marinho e terrestre, respectivamente. As potenciais fontes alimentares foram incorporadas no modelo com base na classificação alimentar dos consumidores (e.g. herbívoro, onívoro) e em estudos de dieta de formigas, aranhas e ratos, disponíveis na literatura (Oelbermann & Scheu 2002, Stapp 2002, Etchian et al. 2016).

Fatores de discriminação trófica (TDF), referidos como ΔN para $\delta^{15}\text{N}$ e ΔC para $\delta^{13}\text{C}$, foram aplicados conforme o tecido analisado de cada consumidor (e.g. sangue total de teiú e fígado de rato) e o tipo de presa inserida no modelo. No capítulo 1, o TDF aplicado para formigas inteiras e músculo de caranguejos foi a média estimada para consumidores generalistas, obtida através de estudo com invertebrados alimentados com plantas e ração proteica (McCutchan et al. 2003). Para aranhas inteiras o TDF utilizado foi baseado nos valores encontrados para aranhas-lobo alimentadas com *Drosophila*

melanogaster (Oelbermann & Scheu 2002). No capítulo 2, o TDF empregado para sangue total de teiú foi obtido com base na média dos valores de TDF encontrados para três espécies de iguanas-das-rochas – *Cyclura* spp. – (Steinitz et al. 2016), e sugerido pelos autores como apropriado para outras espécies de répteis com dieta similar, como é o caso do teiú. O tecido analisado em ratos foi o fígado, por ser um tecido de rápida renovação e, portanto, representar com eficiência a dieta recente. Os valores de TDF para os modelos dos dois capítulos para fígado de rato foram empregados conforme a fonte alimentar (i.e. marinha ou terrestre, com base nos *endpoints* selecionados e indicados no capítulo 1, e itens alimentares – apresentados no capítulo 2. Os valores utilizados foram obtidos através de valores isotópicos de ratos tratados com farinha de peixe, milho, alfafa e caseína (Caut et al. 2008b). Não foi possível realizar análise de isótopos estáveis em tecido de gatos, devido a insuficiência de amostras obtidas ($n = 1$).

Após análises iniciais dos modelos de mistura realizado com várias potenciais fontes alimentares, os itens foram selecionados conforme a menor correlação entre eles, como indicado pelo manual SIAR (Inger et al. 2010). Os itens alimentares selecionados para compor o modelo de mistura no capítulo 2 foram plantas C3 e C4, frutas, formigas, mabuia e ave marinha. Frutas e plantas C3 foram agrupadas, pois apresentam valores isotópicos semelhantes e similaridade ecológica (Phillips et al. 2005). Caranguejos e aranhas-lobo foram retiradas do modelo, pois apresentaram altas correlações com plantas C3/frutas e mabuia, produzindo interpretações ambíguas (Inger et al. 2010).

SÍNTESE DOS RESULTADOS

No capítulo 1, ANOVA e teste *t* relevaram que ao longo da transecção que partiu do ninhal, a variação de $\delta^{15}\text{N}$ em plantas foi melhor explicada pela distância do ninhal do que pela estação do ano. O teste *post hoc* de Tukey indicou variações dos valores de $\delta^{15}\text{N}$ entre os pontos 0 e 10, 0 e 20, 0 e 50 m de distância da área fonte. Porém, os valores de $\delta^{15}\text{N}$ em formigas e aranhas, nos mesmos pontos, foram maiores durante a estação de chuva do que na seca, e não variaram conforme a distância. Análises da transecção da Droost indicaram que os valores de $\delta^{15}\text{N}$ não variaram de forma significativa entre os pontos de coleta para todos os grupos analisados. Contudo, durante o período de chuva, todos os grupos apresentaram maiores valores de $\delta^{15}\text{N}$, com exceção de plantas C3, cujos

valores foram maiores durante a seca. De acordo com os valores de $\delta^{15}\text{N}$ obtidos ao longo da transecção total (i.e. do Dcol até o Droost), foi observada em todos os grupos uma diminuição dos valores de $\delta^{15}\text{N}$ nos organismos, conforme o aumento da distância do Dcol em direção ao Droost.

Análises dos modelos de mistura bayesianos apontaram que todos os consumidores terrestres tiveram contribuição de $\delta^{13}\text{C}$ de origem marinha na síntese de seus tecidos. Com intervalo de credibilidade de 95%, ratos apresentaram maior contribuição de carbono marinho ($\text{CI}_{95\%} = 65\text{--}99\%$) do que terrestre ($\text{CI}_{95\%} = 0\text{--}34\%$) na síntese de tecidos. Aranhas e formigas tiveram contribuições similares entre carbono de origem marinha (30 a 76%) e terrestre (26 a 77%). Ao contrário, caranguejos tiveram maior contribuição terrestre ($\text{CI}_{95\%} = 70\text{--}77\%$) do que marinha ($\text{CI}_{95\%} = 22\text{--}29\%$), a qual representou a menor contribuição marinha na síntese de tecidos entre todos os consumidores analisados.

Os resultados obtidos apontam para a importância dos nutrientes de origem marinha carregados por aves marinhas para a teia trófica terrestre. Esses nutrientes são dispersados horizontalmente de forma uniforme à medida que os organismos se distanciam da fonte, e podem ser detectados até o terceiro nível trófico (dissipação vertical). Os valores de $\delta^{15}\text{N}$ nos tecidos de produtores e consumidores foram maiores durante a estação chuvosa do que na seca (variação temporal), principalmente em indivíduos localizados a mais de 50 m de distância da fonte, indicando ação de fatores físicos na dissipação. Plantas localizadas até 50 m de distância da colônia parecem capazes de manter valores de $\delta^{15}\text{N}$ semelhantes entre as estações devido ao aporte de nitrogênio derivado da colônia presente durante as duas estações. Já em áreas distantes mais de 50 m da colônia, as plantas recebem o aporte de nitrogênio carregado pela chuva, por espécies móveis ou por deposições pontuais (e.g. regurgitados, guano ou carcaças). Além disso, os valores elevados de $\delta^{15}\text{N}$ em plantas C3 durante o período de seca pode estar fortemente atrelado à presença de ninhos de atobás-de-pés-vermelhos. É possível ainda notar a dissipação horizontal de nitrogênio, através da diminuição dos valores de $\delta^{15}\text{N}$ conforme o distanciamento da fonte. Portanto, o enriquecimento da cadeia trófica da ilha por nitrogênio parece ocorrer principalmente através do aporte de nutriente marinho depositado no Dcol (e.g. guano, regurgitados e carcaças). Os valores de $\delta^{15}\text{N}$ encontrados na secção do Droost derivam, além da deposição pontual de nutrientes, da

dissipação de nutrientes provenientes da colônia, realizada pela movimentação dos consumidores e carreado pela chuva.

No capítulo 2, a análise de conteúdo estomacal de 22 teiús indicou alimentação baseada primariamente em frutos, seguido por répteis (e.g. mabuia) e roedores (e.g. *Rattus* spp. e *Mus musculus*). Ratos apresentaram alta contribuição alimentar (%PSIRI) de mabuia. Entretanto, insetos, carrapatos – provavelmente de aves marinhas – e tecido vegetal, também tiveram contribuição na dieta. A análise de 78 amostras de fezes de gatos ferais revelaram 13 itens alimentares distintos. Entre eles a principal contribuição na dieta foi de roedores, aves e répteis. Foram contabilizados 59 indivíduos de mabuia e 44 indivíduos de aves nas amostras de fezes.

A dieta das espécies exóticas também foi inferida através de modelos de mistura a partir das AIE no sangue. Resultados indicaram que para teiú, a maior contribuição alimentar foi dada por frutos ($CI_{95\%} = 62\text{--}79\%$), seguido por insetos, aves marinhas e plantas C4. Mabuia e ratos apresentaram menor importância na contribuição alimentar. As principais fontes alimentares na dieta de ratos estimada por isótopos estáveis foram aves marinhas ($CI_{95\%} = 3\text{--}48\%$), seguida por mabuia e insetos. Frutas e plantas C4 tiveram menor importância. A combinação de métodos de análise de dieta demonstra que espécies exóticas invasoras possuem dietas fortemente dependentes de espécies nativas, endêmicas e ameaçadas de aves e répteis.

CONCLUSÕES

- Através de análises espaciais, temporais e ao longo da cadeia trófica, demonstramos a existência de nutrientes de origem marinha, carregado por aves marinhas, nas teias tróficas terrestres de Fernando de Noronha. O enriquecimento do sistema por $\delta^{15}\text{N}$ se dissipa de forma uniforme, à medida que plantas e invertebrados distanciam-se da área fonte, e pode ser detectado até 100 m de distância.
- Fatores abióticos como a chuva e a movimentação dos consumidores elevam a capacidade de dispersão horizontal do nutriente de origem marinha. A dispersão vertical de nutrientes (i.e. entre níveis tróficos), está associada ao comportamento alimentar dos indivíduos, e contribui com matéria e energia até pelo menos o terceiro nível trófico, representado pelas aranhas nas transecções.
- Consumidores terrestres, incluindo espécies de vertebrados exóticos invasores, apresentam alta contribuição de $\delta^{13}\text{C}$ de origem marinha na síntese de tecidos. Vertebrados exóticos introduzidos em Fernando de Noronha, como gatos, ratos e teiús, possuem dieta altamente dependente de espécies nativas, endêmicas e ameaçadas, como as aves e o lagarto mabuia.
- Aves marinhas são importantes carreadoras de nutrientes de origem marinha para a teia trófica terrestre, e têm sido ameaçadas pelas espécies exóticas invasoras no Arquipélago de Fernando de Noronha. Concluimos que há urgência no estabelecimento de planos de controle para espécies invasoras e a necessidade de planos de manejo para a conservação das aves marinhas e consequentemente do ecossistema terrestre de ilhas oceânicas.

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CAPÍTULO 1

Patterns of seabird-derived nutrient dissipation in terrestrial insular trophic webs

(Manuscrito formatado conforme as regras de envio para a revista *Oecologia*)

Patterns of seabird-derived nutrient dissipation in terrestrial insular trophic webs

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Abstract

The natural flow of organic matter and nutrients usually occurs from land to aquatic environments due to gravity and water flow. However, seabirds reverse the natural flow by bringing out nutrients and energy from oceanic foraging areas to terrestrial breeding areas. Fernando de Noronha Archipelago is the most diverse and important seabird breeding ground in Brazil, where the presence and flow of marine materials towards terrestrial food chain was measured in the present work through stable isotope analysis. A 100 m long transect departing from a masked boobies (*Sula dactylatra*) colony and reaching into a roosting site of red-footed booby (*Sula sula*), was sampled for C3 and C4 plants, ants and spiders during the rainy and dry seasons, and analyzed by Bayesian mixing models to reveal the marine contribution on the terrestrial trophic web. Our hypothesis were: 1) the terrestrial trophic web receives high influence of marine matter brought by seabirds; 2) $\delta^{15}\text{N}$ enrichment dissipates spatially as the distance from source increases, dissipates vertically toward upper food levels, and increase during the rainy season. We found that all terrestrial consumers had variable proportions of marine matter in tissues; in plants, $\delta^{15}\text{N}$ values decreased according to the increase in distance from sources; terrestrial consumers had higher $\delta^{15}\text{N}$ values in the rainy season; marine signatures of $\delta^{15}\text{N}$ were found in organisms up to 100 m from the source, and rain had potential for abiotic dissipation after 50 m from the source during the rainy season. We demonstrated the pathways of nutrient dissipation horizontally, temporally, and along the food chain (i.e. vertical) and the importance of marine-derived nutrient to terrestrial food webs, mediated by seabirds.

Keywords: Allochthonous matter; Lateral transport; Oceanic island; Stable Isotope Analysis; Trophic niche

23 Introduction

24 Seabirds are highly mobile organisms and can carry nutrients and energy between distant or
25 adjacent ecosystems (Korobushkin 2014), such as between oceanic foraging areas and
26 terrestrial breeding areas. Nutrients are introduced on terrestrial ecosystems through guano,
27 regurgitates or carcasses (Anderson and Polis 1998), fertilizing the soil and providing energy
28 for different trophic levels (McLoughlin et al. 2016). On insular terrestrial environments,
29 marine nutrients can be the main source of energy (Korobushkin 2014), modify the dynamics
30 between consumers and resources (Bauer and Hoyer 2014), and connect marine and terrestrial
31 trophic webs (McLoughlin et al. 2016).

32 Seabird colonies provide energy to consumers directly (Polis and Hurd 1995) through
33 eggs, chicks, and carcasses (Sanchez-Piñero and Polis 2000), and indirectly, through nutrient
34 input to food chain by marine debris on the soil, which will be used by producers and
35 detritivorous organisms (Caut et al. 2012). In this systems, low isotopic values of carbon
36 associated with high isotopic values of nitrogen in terrestrial consumers, suggests ^{15}N
37 enrichment from marine resources (Tabak et al. 2016). The intensity of lateral nutrient transport
38 varies according to the productivity of adjacent areas, and usually flows from the most
39 productive towards the less productive environment (Paetzold et al. 2008). Thus, the connection
40 between systems can create dependency of terrestrial species to nutrient inputs derived from
41 aquatic productivity, thus the term marine subsidies (Stapp et al. 1999).

42 Marine nutrients dissipates through several routes in terrestrial environments, varying
43 their distribution patterns. Horizontal variations of nitrogen may occurs along the distance from
44 the source through movement of species as crabs, spiders or ants (Frouz and Jilková 2008;
45 Harada and Lee 2016; Griffiths et al. 2018). Vertical transport may flows among organisms in
46 different trophic levels varying as the complexity of the trophic web (Caut et al. 2012) and
47 nitrogen input on the system (Anderson and Polis 1998). Nutrient dissipation may also vary
48 temporally on the environment, among seasons through abiotic factors such as weather, rain or

substrate type (Li et al. 2017, Rowe et al. 2017), or through life stages of species living there, as seabirds on breeding (Mulder et al. 2011, Stapp and Polis 2003).

The pathways of assimilated carbon and nitrogen in animals could be followed through natural variation in stable isotopes ratios in animal tissues (Tieszen et al. 1983). Because seabird-derived guano increases up to 100 times the availability of nitrogen in environments (Mulder et al. 2011), seabirds are potential nutrient carriers and potentially play a key role in the maintenance of trophic webs in islands. Signatures of $\delta^{13}\text{C}$ in insects can be used to distinguish between aquatic and terrestrial sources on the trophic web (Hyodo 2015). Moreover, invertebrates (e.g. spiders, ants) are the main acceptors of aquatic nutrients on land (Goncharov et al. 2011). Thus, seabird colonies are a great lateral transport study model and stable isotope analysis of arthropods can be used to track the uptake of marine nutrient along terrestrial food webs.

Flows of matter and energy along trophic webs are dissipated in invisible ways, but can be tracked through stable isotope analysis – SIA (Rounick and Winterbourn 1986). Values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are, respectively, 7‰ and 1‰ lowest in the atmosphere than in the ocean (Peterson and Fry 1987). In terrestrial environment, C3 plants have $\delta^{13}\text{C}$ values about -28‰, while C4 plants have values between -14‰ and -10‰. In marine environments, producers at the base of the trophic web have $\delta^{13}\text{C}$ mean values of -21‰ (Peterson and Fry 1987; Fry 2006). Furthermore, $\delta^{15}\text{N}$ values are efficient indicators of trophic levels, due to accumulation of ^{15}N on tissues, which increase from 3‰ to 4‰ in comparison to the previous trophic level (Peterson and Fry 1987). In this context, analysis of isotopic composition in tissues of organisms could provide measures of the presence and relative contribution of marine subsidies in the terrestrial trophic web and on consumer diets (Vander Zanden and Rasmussen 1999). Thus, this analysis can track marine components in spatial/horizontal (i.e. along transects) and vertical (i.e. among different trophic levels), and temporal (e.g. different seasons) dimensions.

Fernando de Noronha Archipelago holds 11 seabird breeding species (Mancini et al. 2016). Among them, four species are listed as threatened in Brazil (MMA 2014). The low reproductive success rates in seabirds, caused by introduced exotic species, among other reasons, is a key factor threatening seabirds worldwide (Russel and Le Corre 2009). In Meio Island in the Archipelago, boobies (*Sula* spp.) as all seabirds carry nutrients and trace elements to soils around their nests (Mallory et al. 2015) where producers and consumers are ^{15}N -enriched (Hobson et al. 1999; Stapp and Polis 2003; Korobushkin 2014). Among exotic species occurring on Fernando de Noronha are black rats (*Rattus rattus*), a species regarded as the main predator of bird nests during breeding seasons elsewhere (Sarmiento et al. 2014; Yang et al. 2014); feral cats (*Felis catus*), which prey upon endemic Noronha skink (*Trachylepis atlantica*), seabirds and terrestrial birds on the Archipelago; and tegu lizards, which also feed on endemic Noronha skink there (Gaiotto et al. chapter 2). In areas of allochthonous input, as Meio Island, consumers can change their food resources and habitat use according to nutrient supplies carried to inland areas (McCaulley et al. 2012; McCaffery et al. 2016; McLoughlin et al. 2016). Understanding patterns of energy and matter flow are key to the proper understanding of connected ecosystems, as well as support measures for the conservation of both marine and terrestrial species in oceanic islands.

The goal of this study was to determine patterns of marine matter dissipation, incorporated by terrestrial organisms, as well as the effects of nutrient transportation mediated by seabirds on terrestrial trophic webs. Thus, we assessed: (i) the presence of marine matter transported by seabirds to terrestrial environments; (ii) the magnitude of spatial (horizontal) dissipation of marine subsidies along the terrestrial environment; (iii) the relative contribution of marine nutrients to tissue synthesis of terrestrial consumers, upward in the trophic web (vertical); (iv) the presence of variation in ^{15}N -enrichment on terrestrial environment and tissues of organisms, among seasons (temporal), and thus the role of abiotic factors (water) on dissipation.

Materials and methods

Study area

Sampling was conducted at Meio Island on Fernando de Noronha Archipelago (3°52'S; 32°26'W), located 360 km from Brazilian mainland, in the equatorial South Atlantic Ocean (Fig. 1). The archipelago has total area of 26 km² and is composed by 21 volcanic islands (Schulz-Neto 2004). The weather is tropical with two well-defined seasons - dry and rainy -, which occur from August to January and from February to July, respectively. Moreover, the mean annual rainfall is 1400 mm (Teixeira et al. 2003). The archipelago is covered by deciduous vegetation, with forest, shrub and herbaceous species (Mello and Oliveira 2016). However, the current vegetation in the archipelago, composed by 331 vascular plants, had been severely altered in comparison to original characteristics, i.e. before human settlement (Alves 2006). Essentially, part of the native vegetation had been removed and invasive species were introduced when the Archipelago was a prison and, later on, by residents (3000 permanent inhabitants) and tourists (2000 each month) (Serafini et al. 2010; IBGE 2017).

Despite pressure, the Archipelago have the highest diversity among Brazilian oceanic islands (Serafini et al. 2010) and is regarded as the most important breeding ground for seabirds, in number of species (11 spp.) and abundance (Mancini et al. 2016). The Meio Island belongs to protected and uninhabited areas, which are managed by Fernando de Noronha Marine National Park. This island holds breeding colonies of masked boobies (*Sula dactylatra*), during both rainy and dry seasons, and red-footed boobies (*Sula sula*), which breed there during the dry season, but roosts during the day and night throughout the year. The island also holds the yellow-crab, *Johngarthia lagostoma*, a Brazilian threatened species that occurs exclusively in Atlantic oceanic islands (Hartnoll et al. 2006), as well the endemic Noronha skink and invasive black rats.

124 Sampling

125 Samples were collected in October 2016 and April 2017 during the dry and rainy seasons,
 126 respectively. A 100 m long transect was sampled, as adapted from Korobushkin (2014). This
 127 transect had two sections, both starting from areas with potential marine nitrogen input, a
 128 breeding site of masked boobies (hereafter Dcol) and a seabird roosting site (hereafter Droost),
 129 respectively (Fig. 2). During the rainy season, red-footed boobies use Droost during daylight,
 130 but more intensively as a nocturnal roosting site. In contrast, there are a few nests of red-footed
 131 boobies during the dry season on the same place. Sampling occurred in points along both
 132 sections, located from 0, 5, 10, 20 and 50 m from the nitrogen input areas (i.e. Dcol and Droost).
 133 The start point at 0 m departed from inside the Dcol, and the same point in the other section
 134 was placed inside Droost. Thus, the midpoint of the whole transect was at 50 m from both areas
 135 with potential nitrogen input (Fig. 2).

136 Plant leaves of C3 (*Ipomoea piurensis* and *Ipomoea alba*) and C4 (*Paspalum*
 137 *pleostachyum* and *Cyperus atlanticus*) photosynthetic pathways were collected manually in
 138 each distance point. Three pitfall traps were installed in each point, in order to capture ants and
 139 spiders for SIA. Pitfalls were checked daily or every other day, always in the morning, and
 140 captured individuals were stored in vials with 70% ethanol. Arthropods were identified by
 141 experts, and whole body of wolf spiders (Lycosidae) and fire ants (*Solenopsis* genus) were used.
 142 The choice of taxa was based on high abundance, great mobility, and food behaviors, i.e.
 143 generalists as fire ants, and carnivorous as wolf spiders. Thus, a trophic chain model with three
 144 levels (i.e. plants as producers, ants as primary consumer and spiders as secondary consumer)
 145 was built. Plant samples were sun dried, stored in ziplock plastic bags and identified by experts.

146 To assess the presence of marine carbon on the diet of terrestrial consumers other than
 147 invertebrates described above, muscle of yellow-crab and liver of black rats were used. Muscle
 148 samples of yellow-crabs were obtained through non-lethal sampling, removing a pereopod,
 149 while rats were trapped with handnet and Tomahawk traps (50 × 21.5 × 20 cm), euthanized and

necropsied. All samples were stored in 70% ethanol due to inability to keep them freezing, however the storage does not result in changes on $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in stable isotope analysis (Hobson et al. 1997).

Preparation and Stable Isotope Analysis (SIA)

Plant samples were oven-dried for 48 h at 60°C, macerated with mortar and pestle, weighted (3 mg) and stored in tin capsules. Spiders were macerated individually in order to obtain mean and standard deviation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, while whole body of ants were pooled to reach minimum amount for SIA due to small body sizes. Lipids from muscle and liver samples were extracted using Soxhlet apparatus with petroleum ether as solvent in 6 h cycle (Mancini and Bugoni 2014). Then, animal samples were lyophilized, homogenized, weighed (1 mg) and stored in tin capsules. All samples were sent to SIA through an Isotope Ratio Mass Spectrometer (IRMS) coupled to an elemental analyzer, on the Center for Stable Isotopes at University of New Mexico (UNM–CSI). Results were accurate at 0.08‰, measured as the standard deviation for $\delta^{13}\text{C}$, and 0.03‰ for $\delta^{15}\text{N}$, based on several measurements of laboratory internal standards with known isotopic values of soy, casein, whey protein and tuna. The international standards used for carbon and nitrogen were Vienna PeeDee belemnite and atmospheric air, respectively, and the isotopic values were expressed by δ in parts per thousand (‰), according to eq. (1) by Bond and Hobson (2012):

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N}(\text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \quad \text{eq. (1)}$$

where, R is the isotopic ratio which represents $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ of $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$, respectively.

Data analysis

Nutrient dissipation from the two potential nitrogen sources were analyzed separated in each 50-m section, i.e. departing from the Dcol and the Droost. Ants and spiders could not be obtained in the dry and rainy seasons, respectively, both at the 5 m point near the Droost.

The variation in $\delta^{15}\text{N}$ values among C3 plants, C4 plants, ants and spiders, was tested with two independent categorical variables: seasons (with two levels: dry and rainy) and distance from source (with five levels: 0, 5, 10, 20 and 50 m). Normality of data was tested for all analysis through Shapiro-Wilk's test. Values of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were compared according to dry and rainy season through Student's t test with “lm” function on R environment (R Core Team 2014). The distance variable was tested through one-way ANOVA with “aov” function, also on R. The *post-hoc* Tukey test was used to check difference in $\delta^{15}\text{N}$ values among sampling points. Significant differences were defined when $p < 0.05$.

Values of $\delta^{13}\text{C}$ in consumer tissues are similar to the source, and thus it is possible to detect the origin of assimilated carbon in food webs (DeNiro and Epstein 1978). Moreover, $\delta^{15}\text{N}$ values in both producer and consumer tissues increase at each trophic level and thus are suitable for the detection of marine nutrients upward in the terrestrial trophic webs, as well as spatially and temporally (Vander Zanden and Rasmussen 1999). Therefore, both isotopes can allow estimates of the relative contribution of food items on consumer diets and the synthesis of its tissues (Vander Zanden and Rasmussen 1999). Thus, Bayesian mixing models were performed through SIAR package (Parnell et al. 2010) on R environmental (R Core Team 2014) to assess the origin (i.e. marine or terrestrial) and the contribution of distinct sources on the terrestrial trophic webs, using both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to tracked uptake in ants, spiders, yellow-crabs and black rat tissues, and $\delta^{15}\text{N}$ as assimilated by plants. The marine and terrestrial endpoints were isotopic values in blood of masked boobies (from database on Laboratório de Aves Aquáticas e Tartarugas Marinhas – FURG) and C3 plant leaves, respectively. Potential sources used for model analysis as plants, ants and seabirds, were selected through the feeding habits of each consumers (e.g. herbivorous) and previous diet studies (Oelbermann and Scheu 2002; Stapp 2002; Etchian et al. 2016). Trophic discrimination factors (TDF), i.e. ΔN for $\delta^{15}\text{N}$ and ΔC for $\delta^{13}\text{C}$, were applied to each consumer to whole body of ants ($\Delta\text{C} = 0.5 \pm 0.13\text{‰}$ and $\Delta\text{N} = 2.3 \pm 0.18\text{‰}$) and muscle of yellow-crab ($\Delta\text{C} = 0.5 \pm 0.19\text{‰}$ and $\Delta\text{N} = 2.2 \pm 0.30\text{‰}$).

These values were based on estimated averages of generalist consumers (McCutchan et al. 2003) obtained through invertebrates fed with plants and protein grains. The TDF values applied to whole body of spiders were based in values of wolf-spiders fed with fruitflies *Drosophila melanogaster* (Oelbermann and Scheu 2002), where $\Delta C = 0.44 \pm 0.1\text{‰}$ and $\Delta N = 2.16 \pm 0.1\text{‰}$. Finally, analysis using black rat liver, a tissue with rapid turnover rates that can represent recent feeding (Tieszen et al. 1983), had TDF values ($SD \pm 0.1$) applied according to the source, where $\Delta C_{\text{terrestrial}} = -2.78\text{‰}$ and $\Delta N_{\text{terrestrial}} = 2.09\text{‰}$, $\Delta C_{\text{marine}} = -0.84\text{‰}$ and $\Delta N_{\text{marine}} = 1.49\text{‰}$ (Caut et al. 2008).

Results

Analysis of colony section

C3 plants maintained similar values of $\delta^{15}\text{N}$ between dry and rainy seasons along the Dcol transect ($t = 0.837$; $df = 8$; $p = 0.427$). However, these values had significant variation among points at different distances from the source ($F = 4.783$; $df = 4$; $p = 0.050$), decreasing as the distance from colony increased. Significant differences in $\delta^{15}\text{N}$ values occurred between 0 and 50 m. Similar results were obtained for C4 plants samples, with $\delta^{15}\text{N}$ values similar between seasons ($t = 0.027$; $df = 8$; $p = 0.979$) but decreased as the distance increased from the colony ($F = 6.951$; $df = 4$; $p = 0.028$). Significant differences were found between points at 0 and 10, 0 and 20, and 0 and 50 m of distance from the colony (Fig. 3).

Distinct results were obtained for ant and spider samples. Ant $\delta^{15}\text{N}$ values varied between seasons ($t = 2.509$; $df = 8$; $p = 0.036$), similar to spiders ($t = 2.942$; $df = 8$; $p = 0.018$; Fig. 3). Neither group showed significantly different $\delta^{15}\text{N}$ values among sampling distances ($F = 1.293$; $df = 4$; $p = 0.385$ and $F = 0.999$; $df = 4$; $p = 0.490$, for ants and spiders, respectively).

Consistent with $\delta^{15}\text{N}$ values obtained in this study, all groups analyzed showed depletion of ^{15}N in tissues as distances from the colony increased, despite some comparisons had not been statistically significant (Fig. 4).

Analysis of roosting site section

All groups analyzed departing from Droost showed variation in $\delta^{15}\text{N}$ values, influenced by season. During the rainy season, plants ($t = 2.365$; $df = 8$; $p = 0.045$), ants ($t = 2.892$; $df = 8$; $p = 0.020$) and spiders ($t = 2.579$; $df = 8$; $p = 0.032$) had $\delta^{15}\text{N}$ values higher than during the dry season. In contrast, C3 plants had higher values in dry season ($t = 2.672$; $df = 8$; $p = 0.028$). Neither group had significant variations in $\delta^{15}\text{N}$ values related with the distance of sampling points in relation to the Droost source.

Marine contribution to terrestrial trophic chain

Bayesian mixing models showed that all consumers had marine ^{13}C into their assimilated diets (Fig. 5). Marine contribution to spiders was estimated from 30 to 76% (95% credibility interval – CI), with terrestrial contribution from 23 to 69%. Ants had similar results to spiders, with marine contribution $\text{CI}_{95\%} = 26\text{--}77\%$ and terrestrial contribution $\text{CI}_{95\%} = 22\text{--}73\%$. Yellow-crabs presented higher terrestrial than marine contribution, with $\text{CI}_{95\%} = 70\text{--}77\%$ and $\text{CI}_{95\%} = 22\text{--}29\%$, respectively. In contrast, black rats were highly dependent of marine matter in their diets ($\text{CI}_{95\%} = 65\text{--}99\%$), while the contribution of terrestrial sources were lower ($\text{CI}_{95\%} = 0\text{--}34\%$).

Discussion

Vertical, horizontal and temporal analysis in insular terrestrial food webs at Fernando de Noronha demonstrated that marine nutrients carried by seabirds are a key resource to terrestrial ecosystems. This study demonstrated that the input of marine matter on the terrestrial trophic web is mediated by seabirds, derived mainly from Dcol and dissipated as the distance increases, but it is still detectable up to 100 m away (horizontal flow). Marine $\delta^{13}\text{C}$ is detected up to the third trophic level (vertical flow), and is strongly related to food habit of consumers. The mobility of consumers such as crabs, ants and spiders also increase the capacity of marine nutrients to disperse spatially, an effect that also occurs by rain, which disperse nutrients to a

greater extent, over 50 m from the Dcol, and increase the marine matter assimilated by plants and consumers during the rainy season (temporal variation).

Horizontal flow

The decrease of $\delta^{15}\text{N}$ values in C3 and C4 plants as the distance from Dcol increases, and for C3 plants in the Droost section, in contrast to increasing $\delta^{15}\text{N}$ values on Droost transect, suggests that marine nutrients are derived from Dcol and dissipated as the distance increases. The areas of allochthonous input are important sources of global nutrient distribution, and higher nitrogen concentrations are expected nearby (Doughty et al. 2016). Seabird colonies enriched the soil and producers (Mallory et al. 2015) through nutrient inputs on land through guano, regurgitates or carcasses (Anderson and Polis 1998). The input of marine matter on Dcol is higher than Droost, then, the nitrogen added through Droost to the ecosystem is not high enough to dissipate towards the intermediate transect point, i.e. 50 m away. In contrast, the nitrogen inputted through Dcol dissipates beyond the intermediate point, reaching the Droost, at 100 m from Dcol. Nutrients deposited on land needs carriers to reach sessile organisms such as plants. Ants, spiders and crabs are known for mediate the nutrient distribution through foraging and transport of food (Frouz and Jilková 2008; Harada and Lee 2016; Griffiths et al. 2018). Thus, plants 50 m away from colony are more ^{15}N -depleted than plants near source. However, ants and spiders, due to high mobility, can obtain food in distant places, maintaining $\delta^{15}\text{N}$ values similar no matter distances from sources. In other islands with seabird colonies, rats (i.e. mobile organisms) have high marine-derived $\delta^{13}\text{C}$ values up to 200 m from sources (Stapp and Polis 2003), whereas in terrestrial invertebrates marine matter can be detected over 50 m from sources (Korobushkin 2014).

Abiotic factors such as rain can also contribute to energy transfer among organisms in an ecosystem (Caut et al. 2012; Rowe et al. 2017). Nutrient flow and dissipation through leaching had been investigated mainly in hilly tropical rain forests with high annual rainfall, such as from 2500 mm up to 9500 mm (Miller et al. 2001; Posada and Schuur 2011; Rowe et

al. 2017). We suggest that, due to flat terrain on Meio Island (CPRM 2011), the limited annual rainfall of 1400 mm (Teixeira et al. 2003) is not enough to remove nutrients from source sites, allowing the runoff and accumulation of nutrient pools in a larger area around colonies. The combination of abiotic (rain) and biotic (guano) factors can also make unique soils in oceanic islands (Machado et al. 2017) such as ornithogenic soils in Fernando de Noronha Archipelago (Oliveira et al. 2014) created because of interaction between the guano available on the ground and physical-chemical reactions (Schaefer et al. 2004). Thus, similar to carrier organisms, rain is able to run nutrients away from sources, and contribute for horizontal flow of nutrients.

Vertical flow

Food habits are important aspects for the vertical flow, among trophic levels. Ants and spiders had similar marine and terrestrial contribution in their diets. The omnivorous habit of ants, that feed on plants, fungus and animal tissues (Lach et al. 2010), makes the proportion of each source (marine vs. terrestrial) to vary according to food availability. On the other hand, spiders are all predators (Oelbermann and Scheu 2002), but receive energetic inputs similar to their prey sources (DeNiro and Epstein 1978), such as ants and other insects. Yellow-crabs had omnivorous diet, ingesting skinks and bird eggs occasionally (López-Victoria and Werding 2008), but their regular diet is vegetarian, including leaves, fruits, flowers (Etchian et al. 2016), and organic matter in decomposition (author's pers. obs.). Moreover, Yellow-crabs at Meio island uses areas distant from the colony. Thus, terrestrial contribution is higher than marine matter contribution in assimilated yellow-crab muscle. We suggest that marine contribution in ants, spiders and crabs are dissipated indirectly, through enrichment of soil and primary producers (Caut et al. 2012). Then, marine nutrients are only available after passing through lower trophic levels and decomposition. In contrast, rats are known for predating eggs and chicks (Hobson et al. 1999; Stapp 2002; Tabak et al. 2016) and marine contribution assimilated in tissues is clearly marked in SIA, which demonstrate that rats receive marine nutrients directly

(Polis and Hurd 1995), by feeding in high trophic levels (e.g. seabird chicks and eggs, and regurgitated fish).

Seabird colonies can increase available nitrogen on terrestrial, or adjacent freshwater trophic webs through guano deposition (Anderson and Polis 1998). Effects of seabird guano are usually higher than inputs mediated by other animals, such as sea turtles in nesting beaches (Bouchard and Bjorndal 2000). In ecosystems with marine inputs, isotopic values in different trophic levels are altered as the food source of seabirds changes (Caut et al. 2012). Our results suggest that besides nutrients carried by seabirds being relevant to enrichment of soil and primary producers (McLoughlin et al. 2016), nutrients can be detected and contribute energetically at least up to the third trophic level (i.e. secondary consumers).

Temporal variation

Masked booby colonies at Fernando de Noronha are maintained in both rainy and dry seasons, and thus provide year-round nutrient input, mainly for soil and primary producers around (Caut et al. 2012; Mallory et al. 2015). Thus, when comparing $\delta^{15}\text{N}$ values of plants among seasons, those located up to 50 m from Dcol maintain similar values, while those in areas over 50 m from Dcol presented higher $\delta^{15}\text{N}$ values during the rainy season than the dry season. Then, the soil and primary producers up to 50 m from Dcol receive nitrogen inputs derived from masked booby colonies, regardless of abiotic factors such as rain. However, over 50 m from Dcol, sessile organisms may depend on season, as they receive nutrients mainly through rain, but also by carriers (e.g. crabs) or local depositions in the short (e.g. regurgitates, guano or carcass) or long-term (ornithogenic soils). High values of $\delta^{15}\text{N}$ in C3 plants on the dry season across the Droost transect may have occurred due to an input inside the roosting site, at 0 m, which may have been caused by the presence of some nests of red-footed boobies. Consumers, such as ants and spiders, can expand their trophic niches when marine input increase in an ecosystem (Recalde et al. 2016). Nutrient pools, produced through rain runoff, provide nutrients to several organisms (Li et al. 2017) even if they are distant from the source. The high availability of ^{15}N -

enriched food may have increased $\delta^{15}\text{N}$ values in consumers during the rainy season, due to similar values between prey and consumers (DeNiro and Epstein 1978). Marine nutrients in terrestrial ecosystem increase the primary productivity benefit the base of trophic chain (McLoughlin et al. 2016; Shatova et al. 2017) and low trophic levels, such as primary consumers. Whereas the use of different food sources is one of the main aspect for the coexistence among species (Rosenzweig 1981), this local high input of resources increases the stability on systems and allows species coexistence by sharing abundant food (Mellbrand and Hambäck 2010). Analysis that experimentally exclude emergent aquatic invertebrates on tropical Brazilian streams, support our results, as the decrease of terrestrial consumers shows that allochthonous input can increase the niche range of predators (Recalde et al. 2016).

Conclusion

In this study we showed that 1) seabirds are important carriers of marine matter, which was deposited on terrestrial environments; 2) the horizontal flow of marine matter on terrestrial ecosystems runs evenly, decreasing as the distance from sources increases; 3) the relative contribution of marine nutrients on the terrestrial trophic web occurs according to food habits of consumers, contributing both directly and indirectly to distinct trophic levels; and 4) the ^{15}N enrichment is higher during the rainy than the dry season, mainly to consumers, and rain contributed to nutrient dissipation to a greater extent.

In terrestrial environments, nutrient flows are benefited by allochthonous input, high productivity, carriers and rain (Griffiths et al. 2018; Li et al. 2017; McLoughlin et al. 2016; Moss 2017). Enrichment in ^{15}N on terrestrial trophic chains have been derived mainly by marine nitrogen input into the colony by guano, regurgitates and carcass. Thus, values of $\delta^{15}\text{N}$ obtained on Droost section are derived from input of a nearby colony, through the displacement of consumers or carried by rain. Therefore, marine matter runs up to 100 m from the Dcol and seems to reach the Droost where the marine input mediated by birds is much lower. These

aspects point out for a local input area in the Droost and a more limited horizontal dissipation, in contrast with a wider effect caused by the colony.

Diet studies demonstrate the impact of invasive species on native and endemic species in oceanic islands around the world (Bonnaud et al. 2007; Caut et al. 2008; Tabak et al. 2016). On Fernando de Noronha Archipelago, invasive cats, rats and tegu lizards have diets strongly dependent on threatened birds and endemic lizard (Gaiotto et al. chapter 2). The current study reveals more deeply ecosystem impacts, by showing seabirds as important carriers of marine nutrients, which increase the ^{15}N available on the nutrient poor terrestrial ecosystem and sustain the trophic web. Thus, reduced seabird populations will also limit the effects on terrestrial nutrient poor ecosystems. Finally, we endorse the necessity of management plans to contribute for seabird conservation as they have positive effects on terrestrial ecosystem in oceanic islands.

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Figure Legends

Fig. 1 Study area in Fernando de Noronha Archipelago, Atlantic Ocean, off northeastern Brazil.

The field work was carried on Meio Island, one of 21 islands in the Archipelago

Fig. 2 Illustration of transects performed in order to collect plants and invertebrate samples on Meio Island, Fernando de Noronha Archipelago. Sampling sites are located along a transect departing from the colony (Dcol) and roosting (Droost) sites, potentials sources of nitrogen for plants, and nitrogen and carbon for upper level consumers

Fig. 3 Variation in $\delta^{15}\text{N}$ values in C3 plants and spiders in the rainy and dry seasons (left), and according to increase distances from the colony (right). * indicates significant differences at $p < 0.05$

Fig. 4 Horizontal patterns of nitrogen dissipation on Meio Island during the rainy and dry seasons, for C3 and C4 plants, ants and spiders. The nutrient flow is uniform and $\delta^{15}\text{N}$ values decrease in relation to increase distances from colony (Dcol).

Fig. 5 Bayesian mixing models to assess the relative contribution of marine (Sea) and terrestrial (Land) matters on diets of yellow-crabs, spiders, ants and black rats on Meio Island, Fernando de Noronha Archipelago. Credibility interval was 95%

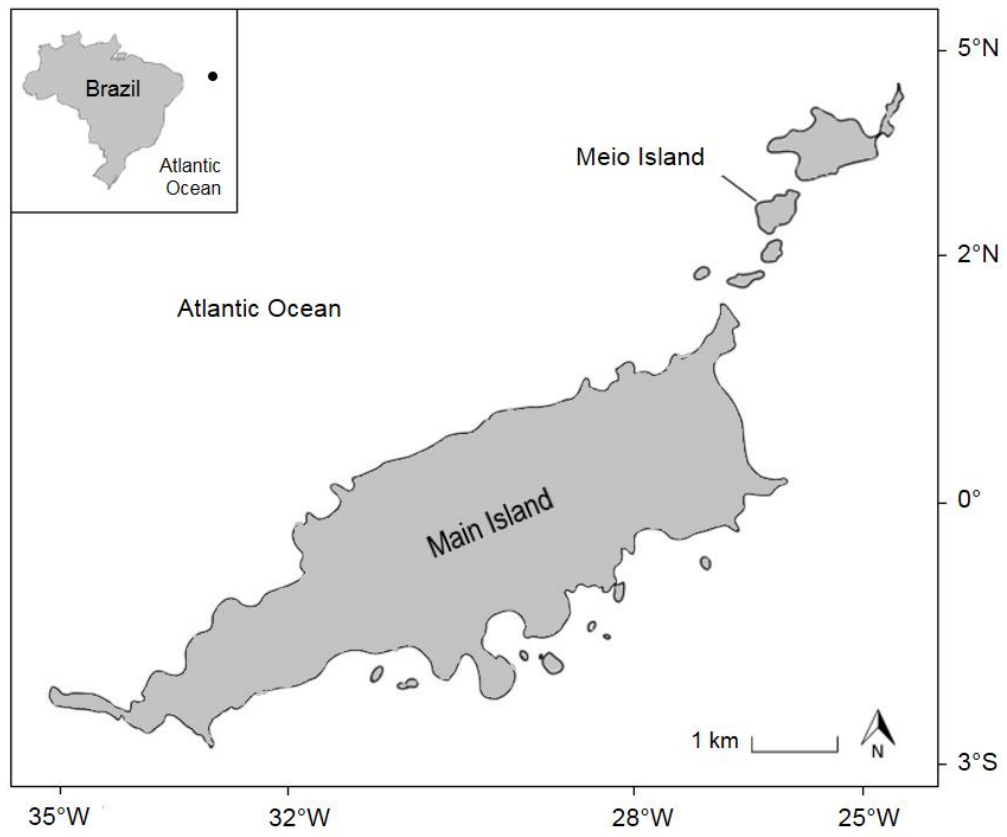
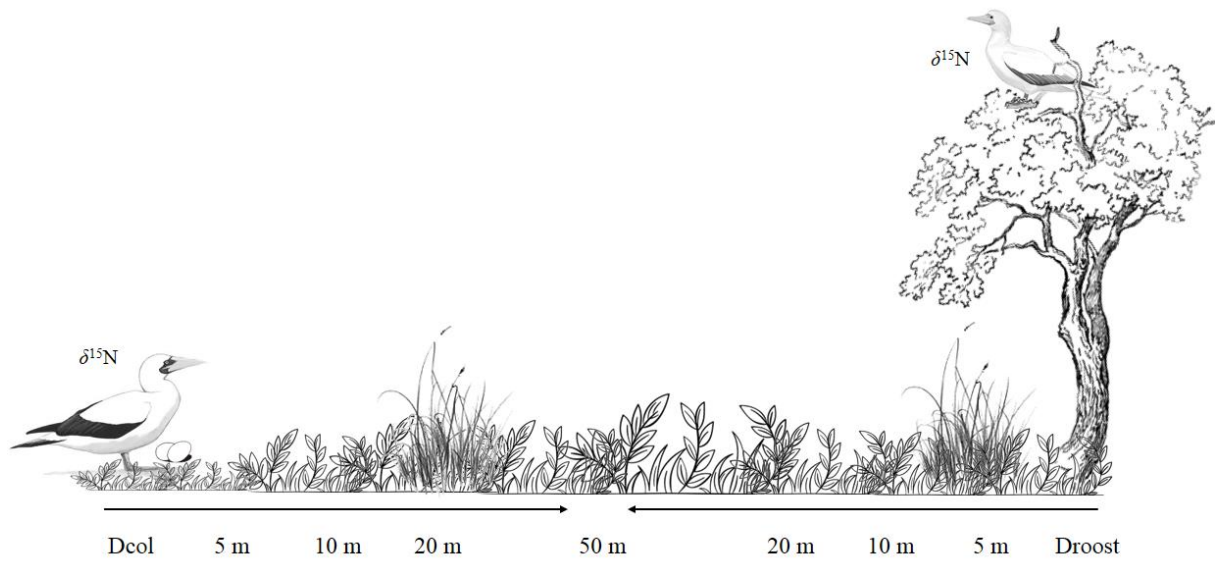


Fig. 1

**Fig. 2**

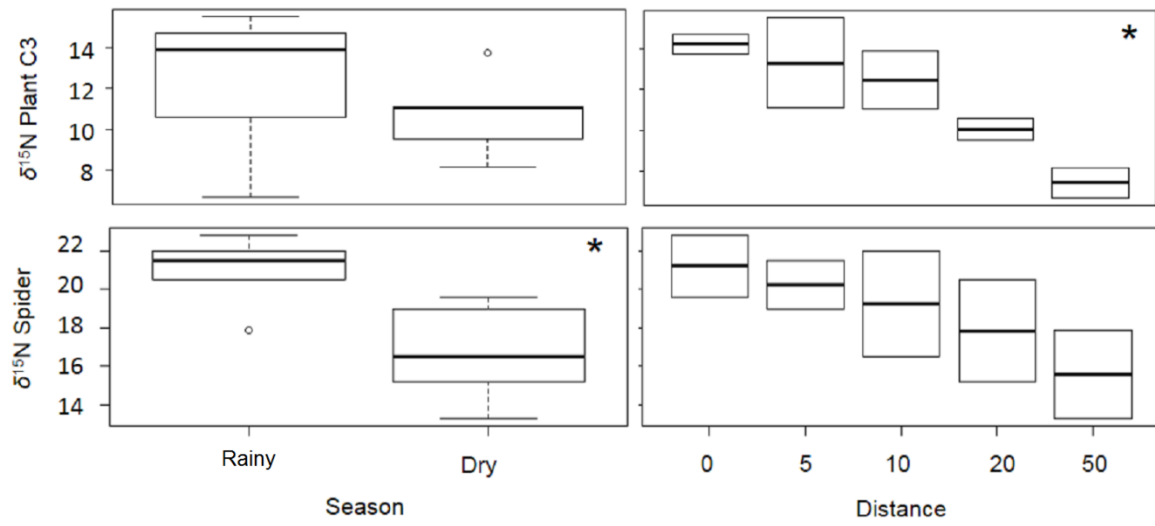


Fig. 3

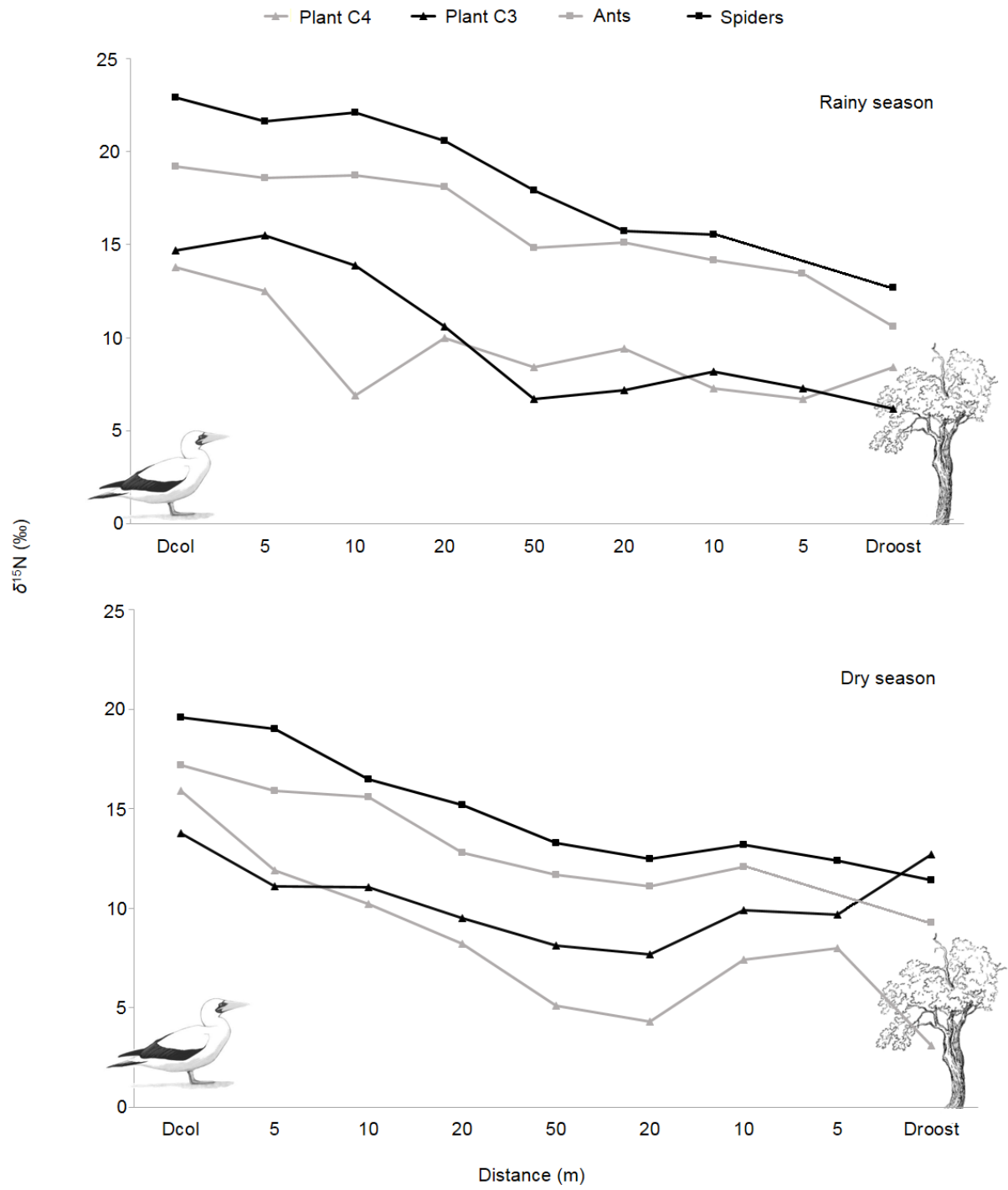
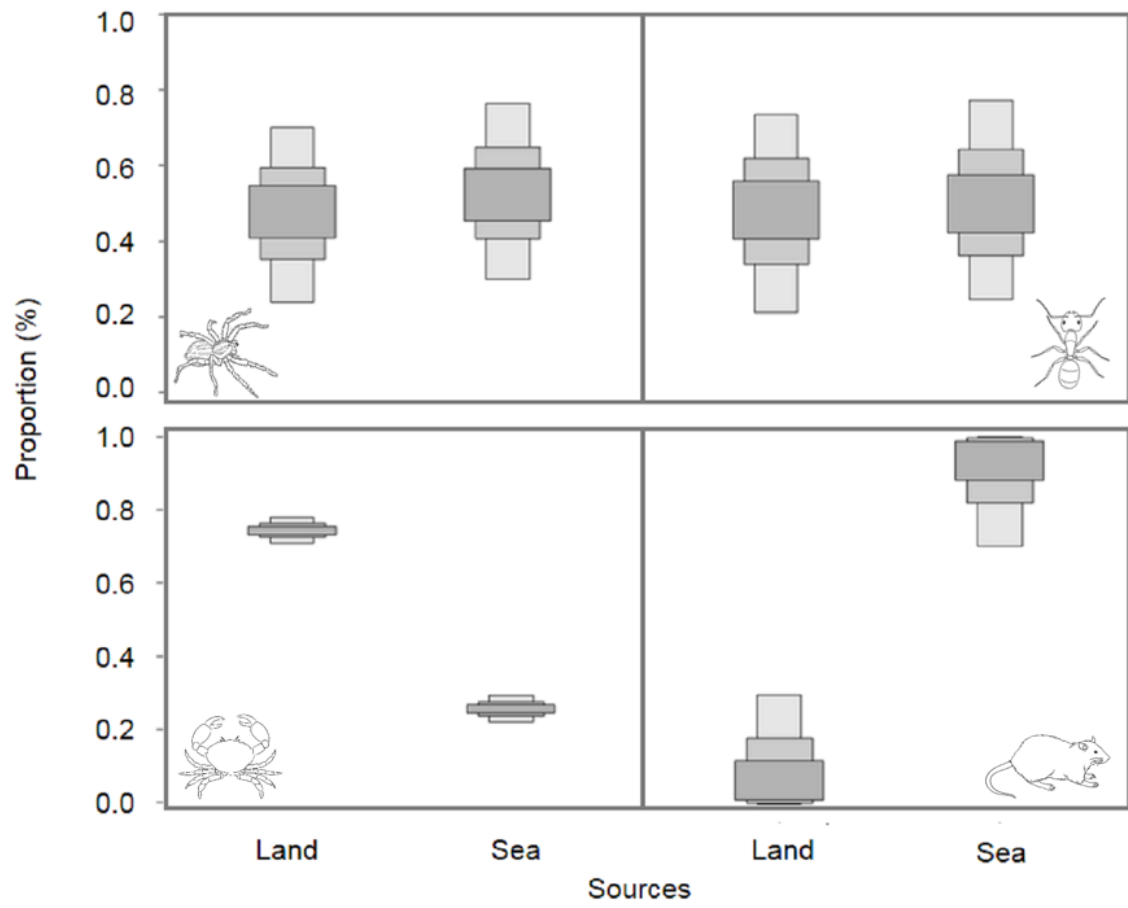


Fig. 4

**Fig. 5**

CAPÍTULO 2

Complementary dietary approaches uncover direct impacts of invasive cats, rats and tegu lizards over threatened species in an oceanic tropical island

(Manuscrito formatado conforme as regras de envio para a revista *Biological Invasions*)

Impacts of invasive cats, rats and tegu lizards over threatened species in an oceanic tropical island are uncovered by complementary dietary approaches

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Abstract

Invasive vertebrate predators have contributed to several extinctions in the modern era, with direct effects mainly over birds, mammals and reptile on islands. Fernando de Noronha Archipelago, in tropical Brazil, is an Important Bird Area (IBA) holding the most diverse breeding seabird community in southern Atlantic Ocean. It is also habitat of a range of endemic birds, reptiles and amphibians. Black rats (*Rattus rattus*), tegu lizards (*Salvator merianae*) and feral cats (*Felis catus*) are among the 26 exotic species reported in the archipelago, all of which are species with potentially high impact over native fauna. Aiming to assess the direct impact of exotic species on endemic fauna, we investigated their diets through stomach content and scats analysis, and indirectly through stable isotope analysis (SIA) and mixing models in tissues of consumers. The main food items for tegu lizards were fruits, with relative importance of 41.3%, and the Noronha skink (*Trachylepis atlantica*; 19.6%); for black rats was Noronha skink (30.3%); and for feral cats diet analysis was composed by rodents (31.6%), birds (28.6%) and Noronha skink (18.8%) as main contributors. SIA provided evidences that marine matter contributed substantially to the synthesis of tissues of all three consumers. This study demonstrated that exotic species are feeding on endemic species in the archipelago. Most heavily impacted species was the endemic Noronha skink. We highlight the importance of protecting endemic species and strongly recommend the developing of a plan for control or eradication of invasive species.

Keywords Management, Seabirds, Skink, Stable Isotopes, Trophic Ecology

Introduction

Invasive predators have contributed to several extinctions in the modern era, the vast majority birds, mammals and reptiles (Doherty et al. 2016). Island ecosystems are particularly vulnerable to the effects of introduced species (Medina et al. 2011), because endemic species lack defensive traits due to their evolution in the absence of natural predators (Banks and Dickman 2007). Invasive species can affect the population structure of native species, modify the community composition and ecological processes through competition, predation or emergence of diseases (Lockwood et al. 2007). Nest predators introduced on islands cause reduction in reproductive success rates in seabirds (Russell and Le Corre 2009; Yang et al. 2014), contributing for almost all recent bird extinctions on islands (Pontier et al. 2008). Introduced rats are the main cause of the high mortality in several bird species, especially at the chick phase (Hobson et al. 1999; Stapp 2002; Jones et al. 2008; Tabak et al. 2016). Chicks of ground and burrow-nesting seabirds such as boobies (*Sula* spp.) and tropicbirds (*Phaethon* spp.), for instance, are strongly affected by rats during the breeding season (Caut et al. 2008a; Leal et al. 2016). Feral and domestic cats are estimated to kill billions of small mammals globally each year (Loss et al. 2013), being responsible for the extinction of 14% of birds and reptiles in islands and for the decline of at least 8% of the all species currently listed as “Critically Endangered” (Nogales et al. 2013). The tegu lizard (*Salvator merianae*) is known to prey upon ground nests (Bovendorp et al. 2008; Mazzotti et al. 2015), where more than a third of bird nests were attacked by tegu lizards in another Brazilian island (Bovendorp et al. 2008).

Islands comprise about 5.3% of the planet terrestrial surface (Tershy et al. 2015). Endemism is characteristic of islands, due to limited distribution ranges and dispersion of species (Kier et al. 2009). Ultimately, the vertebrate endemism richness in islands is eight times greater than in continental regions (Whittaker et al. 2007; Kier et al. 2009), making these sites hotspots of biological diversity. Islands are widely affected by extractive activities, tourism or urbanization (Wetzel et al. 2013), with human occupation closely related to intentional or accidental introduction of exotic species, the main cause of environmental impacts and extinctions on islands (Veitch and Clout 2002; Szabo et al. 2012). Most island extinctions and threats occurred due to direct impact of invasive mammals, with 61% of the species listed as “Extinct” and 37% of those listed as “Critically Endangered” living on these places (Tershy et al. 2015). In a later analysis based on the IUCN Red List (2014) “Endangered” and “Extinct” species added up 81% of insular endemic species threatened by invasive species (Doherty et al. 2016). Moreover, most well-documented bird species extinctions (78.7%) occurred on oceanic islands (Szabo et al. 2012).

Fernando de Noronha Archipelago is an important breeding ground for seabirds, with breeding populations of 11 species (Mancini et al. 2016). Among seabirds that breed on the archipelago, the red-footed booby (*Sula sula*), white-tailed tropicbird and red-billed tropicbird (*Phaethon lepturus* and *Phaethon aethereus*, respectively) are nationally listed as “Endangered” and the Audubon's shearwater (*Puffinus lherminieri*) is listed as “Critically Endangered” due to small population sizes (MMA 2014). The Archipelago also host two endemic reptiles species, Noronha skink (*Trachylepis atlantica*) and Ridley's worm lizard (*Amphisbaena ridleyi*), both listed as “Critically endangered” in the Pernambuco state red list (CPRH 2017), and threatened endemic landbirds Noronha vireo (*Vireo gracilirostris*) and Noronha elaenia (*Elaenia ridleyana*). However, 26 exotic species are listed on the Fernando de Noronha Archipelago (Iabin 2016), including the black rat (*Rattus rattus*), the tegu lizard (*Salvator merianae*) and the feral cat (*Felis catus*), which are the main predators of chicks and bird eggs (Leão et al. 2011).

Black rats have generalist diet and high adaptability to novel environments (Jones et al. 2008). They feed mainly on plants and invertebrates, but often use bird eggs and carcasses as food sources (Stapp 2002). Tegu lizards, native from continental South America, have their diet based on fruits and leaves, but also feed opportunistically on invertebrates, carcasses and animal tissues in general – including birds and other lizards – (Hines 2011; Silva et al. 2014). Cats are opportunistic predators, being active during the day and at night (Dauphiné and Cooper 2009), expanding their predation possibilities. Feral cats feed mainly on rodents, but birds and reptiles are often present in their diet (Bonnaud et al. 2007; Dickman 2009).

Cats in Fernando de Noronha Main island have its population estimated in 1287 (Dias et al. 2017), while tegu lizard population is estimated between 6906 and 12.270 individuals (Abrahão et al. *in press*) individuals on the Fernando de Noronha Archipelago. Cats and rats prey upon the majority of the animal species available in the environment, affecting nesting of seabirds and endemic species where they occur (Bonnaud et al. 2007; Dickman 2009). Rats are among the 100 worst invasive species, as they prey on bird chicks and eggs, occupy the niche of endemic rodents and transmit diseases (Lowe et al. 2000; Major et al. 2007; Caut et al. 2008a). Tegu lizards may have been introduced to Fernando de Noronha Archipelago for “biological control” of rats, but alternatively found easy prey such as endemic Noronha skink (*Trachylepis atlantica*), birds and sea turtle eggs (Schulz-Neto 2004; Leão et al. 2011; Abrahão et al. *in press*), ultimately increasing their population and affecting endemic and resident species.

Invasive species had long been regarded as one of the major threats to biodiversity, with the extinction of species as their main effect (Sampaio and Schmidt 2013). Concurrently, an increased number of plans aiming at eradication in islands as a tool for recovery and maintenance

of populations of native and resident species is being proposed and implemented. Among seabirds that breed on islands, 47% of the species globally listed as “Critically Endangered” and 74% listed as “Endangered” benefited from eradication (Jones et al. 2016). For example, after black rat eradication on the Channel Islands, California, the population of Scripps's murrelet (*Synthliboramphus scrippsi*) triplicated their reproductive success rate (Whitworth et al. 2013), while Langara Island, Canada, was recolonized by Cassin's auklet (*Ptychoramphus aleuticus*) (Regehr et al. 2007). Understanding the impact caused by invasive species upon threatened native species is an important step to raise awareness for conservation, such as population control or eradication.

Effects of exotic species on habitats or over native species are detectable by a variety of ways, such as records of direct predation (Wanless et al. 2007; Dauphiné and Cooper 2009) and niche replacement due to competition, i.e. in breeding or feeding, between exotic and native species (Barbosa-Filho et al. 2009). Indirect effects also include a reduction or destruction of habitats usually by grazers, such as goats, sheep and pigs (Campbell and Donlan 2005; Bengsen et al. 2014), and the dissemination of diseases by rats and feral cats (Magalhães et al. 2016; Silva et al. 2017). Furthermore, aspects of trophic ecology on invasive species can be assessed through conventional diet analysis, using stomach contents or scat samples (Bonnaud et al. 2007), or with complementary methods, using intrinsic markers such as stable isotopes measured in consumer tissues and their food (Galetti et al. 2016). The simultaneous use of different and complementary methods for accessing diet of predators has important benefits. For example, precise estimates in diet analysis of Arctic peregrine falcon (*Falco peregrinus tundrius*) were only possible with motion sensitive cameras as complementary method to stable isotope analysis (Robinson et al. 2017). Results of relative sources contribution in fishes have also more accuracy when isotope methods are combined with trace metals data (Soto et al. 2016). Similarly, the conventional diet analysis also represents a potential complementary method that allows most robust conclusions.

The conventional diet analysis is an important tool for the precise identification of predated species, availability of prey and diet shifts (Sih and Christensen 2001). It is based on the identification and quantification of the content in stomach, feces or regurgitated material, while a limitation is the preferential digestibility of soft items and the predominance of food items with hard and diagnosable parts (Zavala-Camin 1996; Barret et al. 2007). On the other hand, indirect methods for the study of diet include the use of intrinsic markers, such as stable isotope analysis (SIA). This technique allows inferences on the animal feeding in a complementary way to the conventional and direct dietary analysis. In brief, consumers retain in their tissues isotopic signatures of their prey, in a way that $\delta^{13}\text{C}$ isotope values are suggestive of

places where the food was obtained, marked by the photosynthetic characteristics at the base of the food chain. While $\delta^{15}\text{N}$ values increased from 3‰ to 4‰ at each trophic level (Peterson and Fry 1987), and thus indicate the trophic level along the food chain (Fry 2006). In addition, SIA shows food effectively assimilated into animal tissues during a variable period, whereas the traditional diet analysis only shows food items consumed recently, but at a higher taxonomic resolution (Rounick and Winterbourn 1986). Therefore, the simultaneous use of both techniques results in a better resolution of the food chain in a given environment.

Records of predation obtained through dietary studies allow the prioritization of target species directly threatened and which deserve conservation efforts. Before the implementation of management, threatened species must be identified (Burger 2018). The aim of the current study was to determine the main prey items of invasive cats, rats and tegu lizards on Fernando de Noronha Archipelago, focused on the effects upon endemic and threatened vertebrate species. Our results could ultimately support the development of management plans for control or eradication of exotic species in Fernando de Noronha, thus contributing to the conservation of threatened species.

Methods

Study area

Sampling was conducted on Main and Meio Islands, in Fernando de Noronha Archipelago (3°52'S; 32°26'W). The archipelago is in the Equatorial South Atlantic Ocean, 360 km from the mainland (Fig. 1) and has 21 volcanic islands with total area of 18.2 km² (Dias et al. 2017). Climate is tropical with well-defined seasons: dry, from August to January, and wet, from February to July. The only island with permanent human settlement is the Main Island, with available information varying from 2000 to 5000 inhabitants plus up to 3000 tourists each month during the peak season (Andrade et al. 2009; Marinho et al. 2016; IBGE 2017; Pernambuco 2017). The archipelago is inside a National Marine Park in uninhabited areas, while urban areas are within an Environmental Protection Area (EPA).

Sampling

Samples were collected at the Main Island during October 2014, February and November 2015, and February 2016. Tegu lizards were trapped and euthanized using an association of ketamine (20–30 mg/kg) and midazolam (1–2 mg/kg) followed by cerebral perforation, according Brazilian norms and the American Veterinary Medical Association (Leary et al. 2013). The animals were necropsied, blood samples of 29 individuals were collected for SIA and 22 stomach

contents for diet analysis. Blood samples were collected before the administration of the compounds used for euthanasia in order to avoid chemical contamination. These samples were frozen and stomach contents stored in 70% ethanol. At the same island, 78 feral cat scats were collected along tracks in inhabited areas, thus targeting feral cats only, and stored frozen. We were unable to obtain enough blood samples ($n = 1$) to evaluate the diet of feral cats through stable isotope analysis.

Samples of 10 black rats were obtained on Meio Island during October 2016 and April 2017. Rats were trapped at night with handnet and Tomahawk traps ($50 \times 21.5 \times 20$ cm) and euthanized through cervical dislocation in order to avoid chemical contamination on tissues for SIA, according to Brazilian guidelines of Ethics, Bioethics and Animal Welfare Commission (CFMV 2013). Liver, muscle from the femoral region and stomach contents were stored in 70% ethanol.

Sampling of potential food items for SIA was carried out in October 2016 and April 2017. Muscle samples of Noronha skink ($n = 11$) and yellow-crab (*Jhongarthia lagostoma*, $n = 18$) were obtained through non-lethal collection, by tail autotomy and removing a pereopod, respectively. Blood samples of masked booby (*Sula dactylatra*) ($n = 12$) were collected from the tarsal vein with syringe and sterile hypodermic needle in previous studies (Mancini et al. 2014). All animal samples were stored in 70% ethanol due to inability to keep them freezing, however the storage does not result in changes on $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in stable isotope analysis (Hobson et al. 1997). Plant leaves with C3 (*Ipomoea piurensis* and *I. alba*) and C4 (*Paspalum pleostachyum* and *Cyperus atlanticus*) photosynthetic pathways, were collected manually. Samples were sun dried and stored in ziplock plastic bags. Fruits found in stomach contents of tegu lizards such as cashew (*Anacardium occidentale*), in addition to fruits collected on surroundings, as jocote (*Spondias purpurea*) were used to SIA to verify the contribution of fruits to tegu lizard diet.

The procedures were approved by Ethic Committee of the School of Veterinary Medicine of University of Sao Paulo (licences 2724150515, 677813114 and 1827250515) and by the Brazilian Ministry of Environment (SISBio 41682-6, 43589-2 and 22697-7).

Diet analysis

Stomach contents of tegu lizards and black rats were analyzed under stereoscopic magnifying microscope (10×40) and food items identified at the lowest taxonomic level possible. Scat samples of feral cats were washed over a sieve (0.5 mm) under a shower of warm water (Nogales

et al. 1988). All items found such as feathers, bones, fur and seeds were separated and compared to reference material for identification.

Food items were quantified by number (N) and volume (V). Item counting was performed using single, diagnosable food item remains (e.g., mandible, jaw, head) or pairs (e.g., forepaws, hind paws, wings). We used graded glass beaker and glass dish over a graded paper to obtain the total volume of food items (Hellawell and Abel 1971). The length of structures was measured in cubic millimeters on the dish, and converted to ml (i.e., $1 \text{ mm}^3 = 0.001 \text{ ml}$).

Plant samples found on feral cat scats (e.g., grass and seeds) were not included in analysis, as their occurrence is related to accidental intake and do not represent food contribution value.

Stable isotope analysis

Lipids from muscle and liver samples were extracted using Soxhlet apparatus with solvent petroleum ether in 6 h cycle (Mancini and Bugoni 2014). Then, muscle, liver and blood samples were lyophilized, homogenized, weighed (1 mg) and stored in tin capsules.

Sugars were extracted from fruit pulp after centrifugation for 10 min at 4000 rpm and washed with acetone in 50 ml vials (Rossmann et al. 1997). Plants C3 and C4 were oven-dried for 48 h at 60°C. After, plant samples were macerated, weighed (3 mg) and encapsulated as previously described.

All SIA samples were sent to the Center for Stable Isotopes at the University of New Mexico (UNM-CSI) and analyzed in Isotope Ratio Mass Spectrometer (IRMS). Isotopic values were expressed by δ in parts per thousand (‰), according to equation of Bond and Hobson (2012):

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N}(\text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \quad \text{eq. (1)}$$

where, R is the isotopic ratio, which represents $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ of $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$, respectively. Standards used were Vienna PeeDee belemnite and atmospheric air, for carbon and nitrogen, respectively. Standard deviation for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were 0.08‰ and 0.03‰, respectively, obtained after several measures of internal laboratory standards with known isotopic values.

Data analysis

The prey-specific index of relative importance was determined through relative measures to quantification of prey using %PSIRI index (Brown et al. 2011). %PSIRI was calculated using the equation:

$$\%PSIRI = \frac{[(\%PN + \%PV) \times \%FO]}{2} \quad \text{eq. (2)}$$

Measurements used were obtained from the frequency of occurrence (FO), abundance or numerical contribution (N) and volume (V). We obtained the FO from the number of samples containing the selected food item, and the relative occurrence frequency (%FO) is the percentage of FO regarding to the total number of samples analyzed of each consumer species. The N value is obtained counting the number of consumed food item, assessed through each food item that appears on the sample, i.e. number of predated individuals or consumed fruits. The numeric proportion (%PN) is the average of equivalent percentages to N of each sample, excluding those samples in which the food item was absent. Likewise, the total volume (V) of a food item is the sum of volume values obtained in all samples for this item, and the relative volume ratio (%PV) is the percentage of a given item in relation to the total volume of samples in which that item was present.

Bayesian mixing models were used to assess the relative contribution of food items for the diet of each exotic species, through SIAR package (Parnell et al. 2010) on R environment (R Core Team 2014). Potential food sources were selected for the models based on food items found in stomach contents or scats, and previous studies on diet of feral cats (Bonnaud et al. 2007), tegu lizards (Kiefer and Sazima 2002; Castro and Galetti 2004) and rats (Stapp 2002; Caut et al. 2008a). Food to consumer trophic discrimination factors (TDF), denoted as ΔN and ΔC for the $\delta^{15}N$ and $\delta^{13}C$, respectively, were chosen based on consumer tissues, i.e. whole blood for tegu lizard samples, and liver for rat (Caut et al. 2008b; Table 1). The determination of adequate discrimination factors for modeling of terrestrial reptiles usually result in large variations according to taxa (Steinitz et al. 2016), and thus, could lead to biases in mixing models (Bond and Diamond 2011). In addition, there is no values of TDF available in the literature on tegu lizards. Therefore, for whole blood of tegu lizards we used TDF values of $\Delta C = 2.5\text{‰}$ and $\Delta N = 4.1\text{‰}$ (Steinitz et al. 2016), that measured discrimination factors in Rock Iguanas (*Cyclura* spp.), with similar diet as the tegu lizard. In this study, overall TDF across the three iguana species was suggested as suitable for studies with other reptile species (Steinitz et al. 2016).

After initial mixing model runs with a range of food items as potential sources, food items for mixing models were selected according to lowest correlations among them, as indicated in SIAR manual (Inger et al. 2010). We considered the most important items those found in diet analysis, and then, pooled potential food items that presented similar isotopic values and ecology (Phillips et al. 2005). Thus, insect taxon was comprised by two genus of ants (*Solenopsis* and

Camponotus) and C4 plants of two different grass species (*P. pleostachyum* and *C. atlanticus*). Plants with C3 photosynthetic pathway (*Ipomoea* spp.) were pooled with fruits, as they have similar isotopic values. Lizard and seabird taxa were represented by the endemic Noronha skink and masked booby, respectively. The potential food items yellow-crab and wolf spiders were excluded from the final mixing models because they have high negative correlation with important food items, such as fruits and lizards, and had limited contribution to the diet accessed by direct methods.

Results

Diet of tegu lizards

A total of 10 food items were identified in the diet of tegu lizards. The main food item was fruits (%PSIRI = 41.3), which were presented in 77.3% of samples (Table 2), followed by reptiles (%PSIRI = 24.1). In the reptile group, at the species level, the Noronha skink had the largest contribution (%PSIRI = 19.6), with the remaining food items represented by the endemic Ridley's worm lizard (*Amphisbaena ridleyi*) (%PSIRI = 4.55). The Rodents group was composed by exotic vertebrates: rats (*Rattus* spp.), house mouse (*Mus musculus*) and the rock cavy (*Kerodon rupestris*), which contributed together with 14.7% for the %PSIRI and had FO = 22.7%. Lastly, arthropods, including crabs and insects, contributed with 8.7% for %PSIRI to tegu lizards diet (Fig. 2).

Diet of black rats

Animal (%PSIRI = 65.6) and plant (%PSIRI = 34.9) tissues, in a very fragmented state, were identified in the black rat diet. Main food items were the endemic Noronha skink (%PSIRI = 30.3 and FO = 50.0%), with insects and seeds contributing 9.6% and 4.3% for the %PSIRI, respectively (Table 2; Fig. 2). Ticks, probably from seabirds, had %PSIRI = 9.10.

Diet of feral cats

From the analysis of 78 feral cat scats, 13 distinct food items were identified (Table 2). The main food group identified was reptiles, which summed 59 individuals of a single species, the Noronha skink (FO = 62.0%). Birds were represented by 44 individuals with FO = 55.0%, followed by rodents (41 individuals), with *Rattus* and *Kerodon* showing highest occurrence (FO = 18.0% and 19.0%, respectively). %PSIRI demonstrated that rodents contributed with 31.6% in the diet of feral cats, with birds and reptiles contributing 28.6% and 18.8%, respectively (Fig. 2).

Arthropods, including crabs and insects, contributed 13.9%. Other groups had %PSIRI values equal or lower than 2.0%, and included fish, gastropods and cephalopods (Table 2).

Diet inferred by SIA

Mean $\pm$ 1 standard deviation of SI values in whole blood of tegu lizards was $\delta^{13}\text{C} = -22.2 \pm 1.28\text{‰}$ and $\delta^{15}\text{N} = 10.2 \pm 2.05\text{‰}$. The mixing model showed that tegu lizards feed mainly on fruits. This food item contributed from 62 to 79% (95% credibility interval - CI). Insects contributed from 0 to 17%, followed by seabirds and C4 plant tissue with CI from 0 to 16%. Lizards, and rats were less important (CI = 0–13% and CI = 0–14%, respectively; Fig. 3).

Mean SI values in liver samples of black rats were $\delta^{13}\text{C} -20.8 \pm 3.4\text{‰}$ and $\delta^{15}\text{N} = 13.1 \pm 0.8\text{‰}$. The main food items of black rats were seabirds, that contributed from 3 to 48%, followed by endemic Noronha skink, which contributed from 2 to 44% in the diet and insects (CI = 4–41%). Then, fruits and C4 plant tissue had lower importance, with CI from 0 to 30% and from 1 to 30%, respectively, in the diet of black rats sampled (Fig. 3).

Discussion

Based on the combination of classic dietary methods and intrinsic markers as the stable isotopic analysis we demonstrate that exotic vertebrates introduced on Fernando de Noronha have diets strongly dependent on native, endemic and threatened birds and reptiles. SIA provided evidences that marine matter contributed directly or indirectly substantially to the synthesis of consumer tissues, in agreement with %PSIRI values obtained through conventional diet analysis, which indicated food items at a higher taxonomic resolution.

The main food items in tegu lizard diet were fruits and endemic skink, which had higher contribution in conventional diet analysis than estimated by SIA. The endemic Ridley's worm lizard appeared only once in tegu lizard stomach contents. Tegu lizard has an omnivore diet that includes vertebrates, invertebrates and plants (Kiefer and Sazima 2002; Castro and Galetti 2004; Tortato 2007). The significant contribution of Noronha skink on tegu lizard diet was likely due to similarity on their habitats and niches – open habitats with anthropic influence and diurnal habits (Bovendorp et al. 2008; Rocha et al. 2009), and predation facility, because the skinks rest for long periods during the daytime in rocks and leaf litter (Rocha et al. 2009). Low contribution of Noronha skink obtained by SIA could be explained by similar isotopic values of the skinks and insects, two sources that compete in mixing models. The accuracy of SIA is generally restricted to studies in which the diet is derived from sources with large differences in $\delta^{13}\text{C}$

(DeNiro and Epstein 1978), and thus, similar values could result in an artificial increase of insect contribution in the estimation of tegu lizard diet by the mixing models.

Similar results were found in black rat diet analysis, which demonstrated Noronha skink as an important food item in both conventional diet analysis and SIA. Different species of skinks are commonly found in analysis of rat diets living in oceanic islands around the world, and the high contribution of this source had also been demonstrated by SIA. For instance, in Surprise Island, New Caledonia, skinks are heavily consumed by rats during all seasons (Caut et al. 2008a), while in New Caledonia Archipelago, rats consume 23 different skink species, of which six are listed as threatened in IUCN Red List (Thibault et al. 2017). Particularly in tropical oceanic islands, Squamata species are frequently classified in some threat level (Böhm et al. 2013), as they are highly threatened by exotic species mainly when there were no natural predators previous to the invasion (Case and Bolger 1991), as is the case of the skink at Fernando de Noronha.

Seabirds had high contribution in mixing models for rat diet, but not in conventional diet analysis. Some seabird ticks were found in stomach contents, which is an indication of use by rats of marine matter provided by seabirds and their colonies. Rats are able to reduce the complexity of trophic webs and the number of species in the web (Thoresen et al. 2017), as they could shift diet according to the source available (Caut et al. 2008a), thus filling a vast range of ecological niches. In Surprise Island, rats threat different species, according to resources available from different species in each season, alternating between seabirds and sea turtles (Caut et al. 2008a). Results from our SIA support that rats use seabirds as a key resource, through chicks, eggs or carcasses. Then, the absence of birds in our conventional diet analysis does not mean that rats do not eat them, but that were not present in rat diet during sampled season. The fragmented state of rat samples could also have precluded identification. Rats cause deleterious effects on many species living in biodiversity hotspot islands (Harper and Bunbury 2015). In Gough Island, for example, mice cause high mortality of Tristan albatross (*Diomedea dabbenena*) and Atlantic petrels (*Pterodroma incerta*) (Wanless et al. 2007). The competitive use of common resources also affects native species, as demonstrated in the Falkland Islands, where two threatened landbirds – *Troglodytes cobbi* and *Cinclodes antarcticus* – were extirpated by rats due to high overlapping niche among them (Tabak et al. 2016).

Feral cats are strict carnivores and scats had proven that in Fernando de Noronha they prey mainly on rodents, birds and Noronha skink. The primary prey was black rats, probably contributing largely to the persistence and high abundance of feral cats on the archipelago, as also demonstrated in a small Mediterranean island where both species co-occur (Bonnaud et al.

2007). Furthermore, a large proportion of the cat population on Fernando de Noronha is fed by humans, even if they do not own cats (Dias et al. 2017), providing subsidies for feline survival, particularly during periods of food shortage. The high contribution of birds on feral cat diet can be due to seabirds nesting on the ground, in burrows or rock crevices (Leal et al. 2016), which makes them vulnerable to predation. This could have limited the distribution of seabirds in Fernando de Noronha, which currently are restricted to peripheral islands and islets, or over trees and steep cliffs when nesting on the Main island (Mancini et al. 2016). The presence of seabirds on islands support a high complexity of trophic interactions and the predation negatively affects the dynamics of the archipelago community (Thoresen et al. 2017). In addition, the endemic landbirds, Noronha vireo and Noronha elaenia, have high abundances in the National Marine Park on the Main Island (Mestre et al. 2016), which overlap with an area of high density of free roaming cats (Dias et al. 2017), making endemic landbirds easily accessible to cats.

Overall, our results demonstrated that invasive vertebrate species are a threat to endemic vertebrates at Fernando de Noronha Archipelago. The predation by invasive species causes a cumulative predation pressure upon native species (Thibault et al. 2017), which may decline Noronha skink population in the long-term or keep densities much lower than in a natural scenario, as they are regularly preyed by all exotic species analyzed, i.e. cats, rats and tegu lizards.

The situation of landbirds and seabirds, nationally or globally threatened by extinction, is also of concern. The only two endemic landbird species, the Noronha elaenia and Noronha vireo, have small populations (Mestre et al. 2016) and are classified as “Vulnerable” and “Near Threatened” (IUCN 2017), respectively. The red-billed tropicbird breeding on Fernando de Noronha Archipelago has population of only ten individuals (Silva-e-Silva 2008). This population is genetically closely related with those living in Abrolhos, Brazil, but holds less heterozygosity levels (Nunes et al. 2017). Likewise, red-footed booby breeds only on Fernando de Noronha Archipelago, after recent extinction on Trindade Island, Brazil (Mancini et al. 2016). A recent study shows that this population differ genetically from others in the Atlantic Ocean (Morris-Pocock et al. 2016), resulting in high levels of inbreeding. In addition, in Fernando de Noronha red-footed boobies are restricted to areas with trees or cliffs (Mancini et al. 2016), despite invasive species on archipelago also occupy these areas. Audubon's shearwater is listed as “Critically Endangered” in the Brazilian Red List due to breeding sites restricted to Fernando de Noronha Archipelago (Mancini et al. 2016). Moreover, the maximum number of individuals seen in the archipelago was 30 individuals (Silva-e-Silva and Olmos 2010) and population size was estimated as fewer than 50 mature individuals (Lopes et al. 2014). This species had also been

locally extinct in southeastern Brazil, where it had bred in late 1990s (Lopes et al. 2014). Seabirds often have high intraspecific genetic variation (Friesen et al. 2007), which becomes an important step for evolution of populations. Thus, the presence of few individuals, low genetic variation and vulnerability to invasive predators make these populations susceptible to local extinction (Mancini et al. 2016; Nunes et al. 2017).

Similar to the scenario demonstrated in this study, invasive species impact several native and endemic species in other oceanic islands around the world. The eradication has been considered a powerful tool to protect native threatened wildlife (Jones et al. 2016). The eradication can benefit not only target species, but the entire island ecosystem. For example, in Anacapa Island, California, the black rat eradication led to an increase in the endemic deer mice (*Peromyscus maniculatus anacapae*), intertidal invertebrate cover, and the return of storm-petrel (*Oceanodroma homochroa*) and Cassin's auklet to breed there (Newton et al. 2016). Similar to our results in diet analysis, feral cats threaten birds and the endemic lizard (*Urossaurus auriculatus*) on Socorro Island, Mexico. After cat eradication, the island presented increase on lizard and bird populations (Ortiz-Alcaraz et al. 2017). Resident lizards also turn to recover in Korapuki Island, New Zealand, after rat eradication (Monks et al. 2014). These examples on similar islands elsewhere are useful as potential predictors of potential outcomes of mammal eradication on Fernando de Noronha Archipelago.

Usually, exotic species eradication programs are established without knowledge about predation upon native and endemic species. For effective conservation we need identify the more threatened species and their causes, thus reducing risks of extinction (Burger 2018). Defining the main food items in the diet of invasive species is one of the first steps for the development of a management plan. To our knowledge, our study is the first to investigate the feeding patterns of tegu lizard using the conventional methodology associated with SIA evidencing them as a threat to the endemic island species. This study highlights the importance to protect the endemic species that has declined with invasive species activity on Fernando de Noronha Archipelago. We strongly recommend the elaboration and adoption of an action plan to control invasive species in the archipelago, preferentially considering the establishment of an exotic species eradication program.

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Table 1 Trophic discrimination factors used to estimate the assimilated diet of black rats based on stable isotopes. The standard deviation used was 0.1‰ for both carbon and nitrogen, as calculated by Caut et al. (2008b)

Food items	$\Delta C(\text{‰})$	$\Delta N(\text{‰})$
Seabirds	-0.84	1.49
Lizards	-1.77	-0.03
Insects	1.07	0.8
Plant C4	1.21	2.73
Plant C3	-2.78	2.09

Table 2 Diet composition of feral cats *Felis catus*, tegu lizard *Salvator merianae* and black rats *Rattus rattus* in Fernando de Noronha Archipelago, determined through scats and stomach content analysis

Food items	FO	%FO	N	%PN	V(ml)	%PV	%PSIRI
<i>Felis catus</i> (n = 78 scat samples)							
Mammals	45	58	48	39.20	105.52	75.98	35.47
Unidentified mammals	7	9	7	25.30	12.71	58.27	3.87
Unidentified Rodentia	11	14	11	47.00	27.69	91.37	9.95
<i>Rattus</i> spp. (black rat and Norway rat)	14	18	15	45.80	27.64	74.63	11.03
<i>Kerodon rupestris</i> (rock cavy)	15	19	15	34.00	37.48	74.23	10.62
Reptiles	48	62	59	39.10	25.93	21.09	18.85
<i>Trachylepis atlantica</i> (Noronha skink)	48	62	59	39.10	25.93	21.09	18.85
Unidentified birds	42	55	44	39.10	83.42	65.48	28.61
Unidentified fish	3	4	4	45.50	5.30	53.99	2.01
Cephalopoda	1	1	1	14.20	0.009	0.16	0.16
<i>Argonauta</i> sp.	1	1	1	14.20	0.009	0.16	0.16
Unidentified Gastropoda	3	4	6	34.30	0.20	1.58	0.77
Arthropoda	37	48	105	46.00	1.85	3.97	13.99
<i>Goniopsis cruentata</i> (red mangrove crab)	7	9	7	37.30	5.67	24.62	2.80
Lycosidae	1	1	1	20.00	0.12	2.32	0.14

Unidentified Insecta	5	5	5	34.93	0.07	0.56	1.15
Hymenoptera ^a	22	29	82	50.25	0.41	0.66	7.27
Diplopoda	3	4	7	48.61	1.02	29.27	1.52
Coleoptera	1	1	2	25.00	0.20	19.55	0.29
Blatodea	1	1	1	50.00	0.02	0.64	0.33
<i>Salvator merianae</i> (n = 22 stomach samples)							
Mammals	7	31.82	8	40.58	52.64	69.97	17.59
Unidentified mammals	2	9.09	2	17.05	2.34	47.48	2.93
Unidentified Rodentia	1	4.55	1	33.33	2.50	91.41	2.84
<i>Mus musculus</i> (house mouse)	1	4.55	2	100	32.00	100	4.55
<i>Rattus</i> spp. (black rat and Norway rat)	2	9.09	2	41.67	6.80	64.23	4.81
<i>Kerodon rupestris</i> (rock cavy)	1	4.55	1	33.33	9.00	75.00	2.46
Reptiles	8	36.36	8	66.76	19.44	65.74	24.10
<i>Trachylepis atlantica</i> (Noronha skink)	7	31.82	7	62.01	18.72	60.85	19.55
<i>Amphisbaena ridleyi</i> (Ridley's worm lizard)	1	4.55	1	100	0.72	100	4.55
Arthropoda	7	31.82	8	37.12	7.26	17.30	8.67
<i>Goniopsis cruentata</i> (red mangrove crab)	2	9.09	2	50.00	6.60	21.04	3.23
Unidentified insects	2	9.09	2	41.67	0.27	31.16	3.36
Homoptera	1	4.55	2	18.18	0.07	2.01	0.46

Blatodea	2	9.09	2	29.17	0.31	6.37	1.62
Plant tissue	17	77.27	21	53.69	131.64	53.25	41.32
Unidentified plant	1	4.55	1	25.00	0.07	3.20	0.64
Seeds	5	22.73	5	45.15	3.12	42.51	9.96
Fruits ^b	11	50.00	15	60.19	128.44	62.69	30.72
Unidentified material	4	18.18	4	52.27	0.79	52.27	8.33
<i>Rattus rattus</i> (n = 10 stomach samples)							
Animal tissue	9	90	58	50.31	10.18	39.23	65.56
Unidentified animal tissue	4	40	3	34.78	3.72	49.07	16.57
Reptiles	5	50	5	47.38	5.04	74.57	30.28
<i>Trachylepis atlantica</i> (Noronha skink)	5	50	5	47.38	5.04	74.57	30.28
Arthropoda	6	60	50	60.52	0.34	3.23	18.71
Unidentified insects	4	40	4	45.83	0.27	3.26	9.61
Ticks	2	20	46	89.91	0.06	3.18	9.10
Plant tissue	7	70	8	36.95	6.86	51.43	34.94
Unidentified plants	6	60	6	40.21	5.84	62.61	30.64
Seeds	2	20	2	27.17	1.02	17.90	4.30

^a Hymenoptera group represents several species of ants.

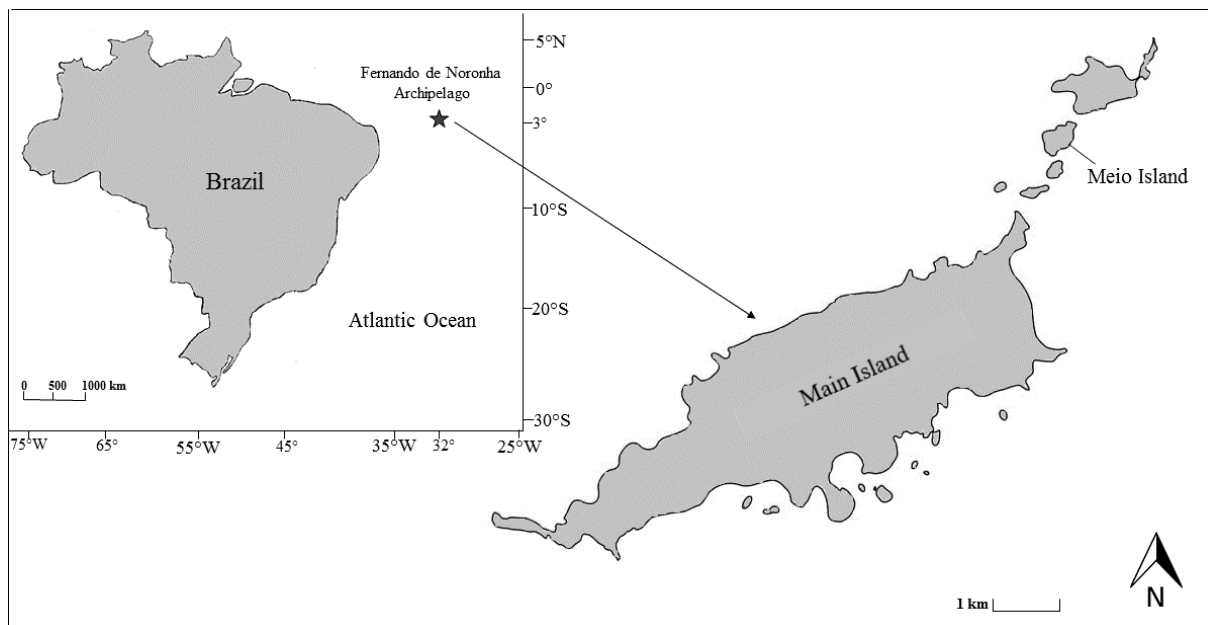
^b Fruits group include tropical fruits like cashew, jocote, watermelon, pineapple and banana.

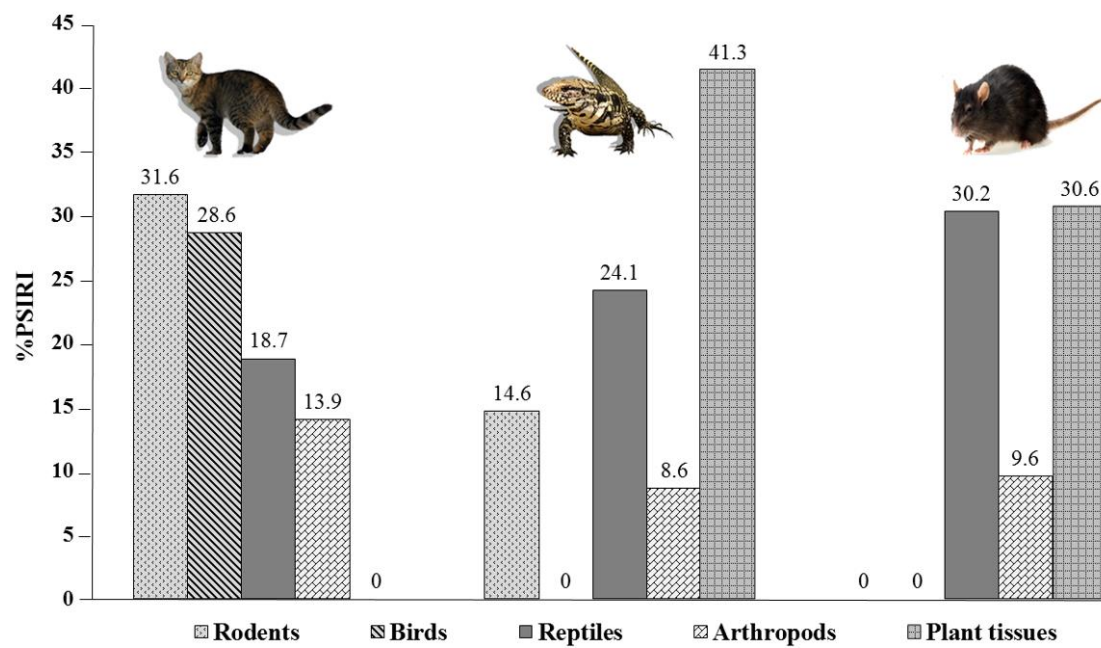
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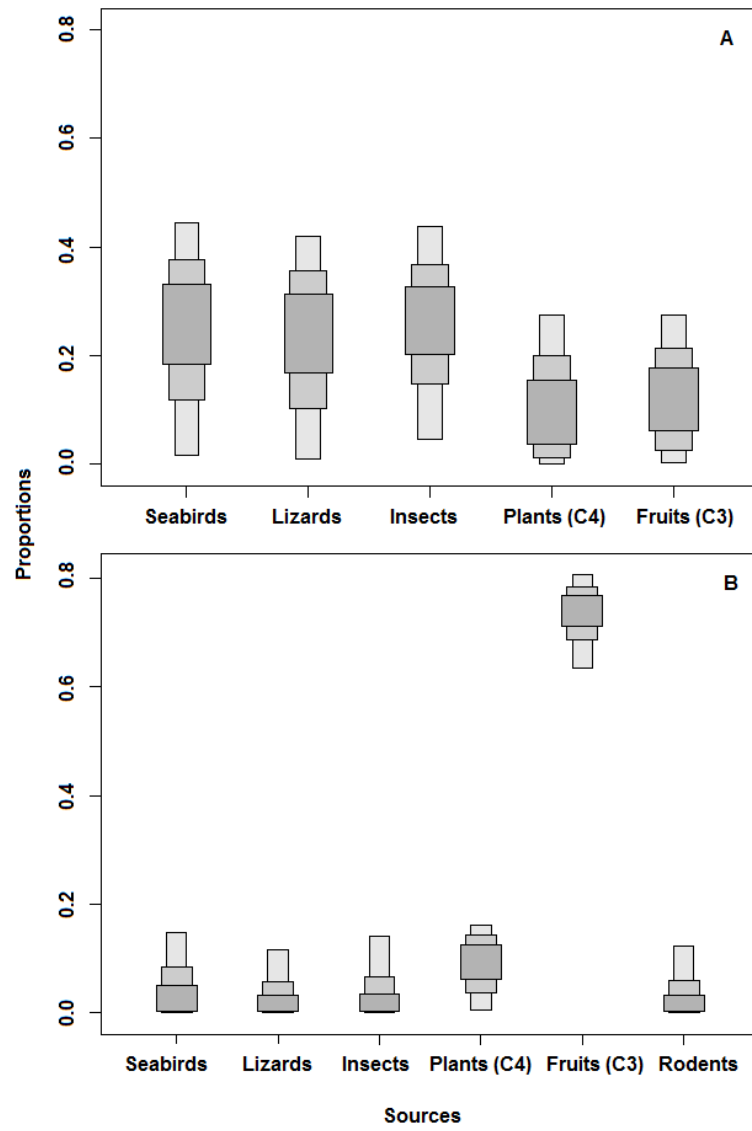
Fig. 1 Study area in Fernando de Noronha Archipelago and the two islands where de field work was carried out (Main and Meio Islands)

Fig. 2 Prey specific contribution of the main food items to the diet of feral cats, black rats and tegu lizards in Fernando de Noronha, Brazil

Fig. 3 Proportions of the food items in black rats *Rattus rattus* (A) and tegu lizards *Salvator merianae* (B) diets in Fernando de Noronha Archipelago, estimated by stable isotope mixing models. Results were obtained from 10 liver samples of rats and 29 blood samples of tegu lizards. Sources were based on food items found in stomach contents

**Fig. 1**

**Fig. 2**

**Fig. 3**