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INFLUÊNCIA DAS CARACTERÍSTICAS AMBIENTAIS SOBRE A ESTRUTURA POPULACIONAL DE *Sula leucogaster* NO BRASIL

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Dedico esta tese àqueles dispostos a encarar os desafios de entender a vida...

Que sirva de inspiração!

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"*Nothing in biology makes sense except in the light of evolution*"

(Theodosius Dobzhansky)

ÍNDICE GERAL

LISTA DE ANEXOS

RESUMO

A associação de gradientes ambientais com a distribuição espacial dos organismos permite compreender o papel da ecologia sobre os processos evolutivos, e vice-versa. No entanto, a identificação de tais padrões em predadores de topo marinhos é dificultada pela alta mobilidade desses organismos e pela inerente dificuldade de caracterizar o altamente dinâmico ambiente oceânico. As aves marinhas, embora capazes de transpor grandes distâncias em curto tempo e sobrepor a área de vida de populações adjacentes, apresentam alta filopatria natal e reprodutiva, ou seja, baixas taxas de dispersão, lançando luz sobre os fatores que influenciam a diferenciação populacional e a promoção de diversidade biológica no grupo. O atobá-marrom, *Sula leucogaster*, é uma ave estritamente marinha, distribuída em todos os oceanos e com forte estrutura filogeográfica. No presente estudo, foram utilizadas seis colônias da espécie distribuídas ao longo de um gradiente latitudinal de 0 a 27°S na região sudoeste do Oceano Atlântico, para avaliar o papel das variáveis ambientais e características intrínsecas de cada colônia como pressões de seleção sobre a diversidade fenotípica e genotípica. Para isso, foram utilizados dados morfométricos e de nove *loci* de microssatélites para investigar diferenças entre populações, além de dados de rastreamento remoto, de isótopos estáveis, e de sistema de informação geográfica, para identificar padrões de variabilidade fenotípica intrapopulacional relacionados ao comportamento de forrageio, à dieta, e à qualidade do ninho. Dados físicos obtidos de imagens de satélite e boias oceanográficas, como temperatura do ar, temperatura superficial do mar, e concentração de clorofila α também foram utilizados como variáveis explicatórias. Os atobás-marrons do Oceano Atlântico sudoeste apresentaram tamanho e massa corporais relacionados com a temperatura do ar, consistentemente com a Regra de Bergmann, exceto pelo desvio do padrão observado na população mais ao norte

(Arquipélago de São Pedro e São Paulo), a qual possui características ambientais peculiares, como pequena área, alta densidade demográfica, e abundante oferta de alimento. Essa população também esteve isolada geneticamente das demais, as quais dividiram-se em mais dois grupos, sendo um relacionado às águas pelágicas pouco produtivas (Arquipélago de Fernando de Noronha e Atol das Rocas), e outro relacionado às aguas ricas em nutrientes sobre a plataforma continental (Arquipélago de Abrolhos, Ilhas Cagarras e Ilhas Moleques do Sul). Por fim, foi demonstrado que os atobás-marrons da colônia de São Pedro e São Paulo, os quais foram demonstrados ser geneticamente e fenotipicamente distintos dos demais, têm como principal pressão seletiva de tamanho corporal a qualidade do ninho. Pelo fato da área útil para reprodução ser bastante reduzida, ninhos de baixa qualidade podem comprometer o sucesso reprodutivo por conta da inundação, destruição por ondas, ou predação, e, portanto, é sugerido que o maior tamanho corporal de fêmeas, as quais conquistam e defendem os territórios, seja vantajoso naquela população, elevando a média de tamanho corporal de atobás-marrons de São Pedro e São Paulo em comparação com outras colônias. Em resumo, a adaptação às condições ambientais locais parece influenciar as taxas de dispersão de atobás-marrons mais do que a distância geográfica e, portanto, o modelo de Isolamento pelo Ambiente explica melhor a estrutura populacional de atobás-marrons no Brasil. Com isso, o presente estudo demonstra que a estrutura populacional de predadores de topo marinhos pode ser altamente dependente das condições ambientais, demonstrando a suscetibilidade da biodiversidade marinha frente às mudanças climáticas.

Palavras-chave: aves marinhas, comportamento de forrageio, dieta, isolamento pelo ambiente, isótopos estáveis, rastreamento remoto, Regra de Bergmann, variabilidade fenotípica.

ABSTRACT

The association between environmental gradients and the spatial distribution of organisms allow us to understand the role of ecology in evolutionary processes, and vice versa. However, the identification of such patterns in marine top predators is hampered by the high mobility of these organisms and the inherent difficulty of characterizing the highly dynamic oceanic environment. Seabirds, although capable of travelling large distances in a short time and overlapping home ranges with adjacent populations, present high natal and breeding philopatry, that is, low dispersal rates, shedding light on the factors influencing population differentiation and promoting biological diversity in this group. The brown booby, *Sula leucogaster*, is a strictly marine bird occurring in all ocean basins and with strong phylogeographic structure. In the present study, six brown booby colonies distributed along a latitudinal gradient of 0−27°S in the southwestern Atlantic Ocean were used to assess the role of environmental variables and colony-specific characteristics as selective pressures on the phenotypic and genotypic diversity of this species. For this, morphometrics, stable isotopes, demographic, and microsatellite data were used to investigate interpopulation differences, in addition to remote tracking, stable isotopes, and geographic system to identify patterns of intrapopulation phenotypic diversity in relation to foraging behavior, diet, and nest quality. Furthermore, environmental variables obtained from satellite images and oceanographic buoys were used, such as air and sea surface temperature, and chlorophyll α concentration. Brown boobies presented body mass and size correlated to air temperature, consistent to the Bergmann's rule, except for the population from Saint Peter and Saint Paul, which has very peculiar environmental characteristics, such as small area, high demographic density, and abundant food supply. Boobies from Saint Peter and Saint Paul were also

genetically isolated from the remaining colonies, which were further divided into two clusters: the first related to oligotrophic waters (Fernando de Noronha Archipelago and Rocas Atoll), and the second related to nutrient-rich waters over the continental shelf (Abrolhos, Cagarras, and Moleques do Sul Archipelagos). Finally, it was demonstrated that obtaining and defending high quality nests may be the main selective force shaping body sizes of brown boobies breeding in Saint Peter and Saint Paul in comparison with other colonies. Due to the reduced nesting area, low quality nests tend to fail during breeding activities by flooding, destruction by waves, or predation, and thus it is suggested to be advantageous that females have large body sizes, since this gender is responsible for obtaining and defending territories. In summary, adapting to local environmental conditions seems to influence dispersal rates in brown boobies rather than geographical distance and, therefore, the Isolation by Environment model explains better the population structure of brown boobies in Brazil. Thereby, the present study demonstrates that population structure of marine top predators can be highly dependent on environmental conditions, illustrating the sensitivity of marine biodiversity to climate changes.

Key words: Bergmann's rule, diet, foraging behavior, isolation by environment, phenotypic diversity, remote tracking, seabirds, stable isotopes.

LISTA DE TABELAS

Anexo 1

Anexo 2

Anexo 3

LISTA DE FIGURAS

Anexo 1

Anexo 2

Material Suplementar 6. Importâncias das variáveis dependentes em modelos rodados com 1000 árvores de regressão, e 20 permutações por árvore para dados *out-of-bag*, através do algoritmo de Floresta Aleatória. %IncMSE representa o quão pior o modelo fica sem cada uma das variáveis, de modo que as variáveis mais importantes são caracterizadas pelas maiores diminuições na acurácia do modelo (variáveis no topo). IncNodePurity mede o quão puros os nós são no fim da árvore. Carbono = $\delta^{13}C$; Nitrogênio = $\delta^{15}N$; D = distância total percorrida; Dmax = máxima distância da colônia; T = duração da viagem; Sinuosidade = $Dist*2Dmax^{-1}$.....**232**

INTRODUÇÃO

Compreender o papel da heterogeneidade ambiental sobre a configuração da biodiversidade, sob a ótica da ecologia evolutiva, é um elemento chave para o estudo de qualquer organismo em qualquer ecossistema (Richardson *et al*., 2014). A identificação de associações da diversidade biológica com pressões de seleção revela os processos microevolutivos agindo sobre as formas existentes e viabiliza prever como as populações naturais responderão às mudanças climáticas (Hoffmann & Sgrò, 2011). De modo geral, gradientes ambientais baseados em latitude, longitude, altitude, profundidade, temperatura, dentre outros, têm sido sugeridos como promotores de distribuição espacial da biodiversidade, auxiliando no refinamento das fronteiras biogeográficas (Cox *et al*., 2016) e na discussão de novidades no campo da filogeografia (Avise, 2000).

Quando os exemplos e tendências são mais comuns do que as exceções, tais padrões podem ser considerados regras ecogeográficas (Gaston *et al*., 2008). Entre as regras ecogeográficas mais conhecidas está a Regra de Bergmann, a qual sugere relação inversa do volume corporal com a temperatura do ambiente (Bergmann, 1847). Em outras palavras, a regra sugere uma tendência de que indivíduos com maior tamanho corporal sejam encontrados em ambientes com menor temperatura (e, portanto, em maiores latitudes) por conta da menor dissipação de calor interno, uma vez que a área corporal cresce em proporção menor do que o volume corporal (Figura 1; Mayr, 1956). Esse padrão tem sido demonstrado em diversos grupos animais, como mamíferos (Clauss *et al*., 2013; Martinez *et al*., 2013), aves (James, 1970; Ashton, 2002), e até mesmo em ectotérmicos, como lagartos (Angilletta-Jr. *et al*., 2004). No entanto, há discussões sobre

a terminologia empregada em relação ao nível taxonômico ao qual a regra se refere (Blackburn *et al*., 1999).

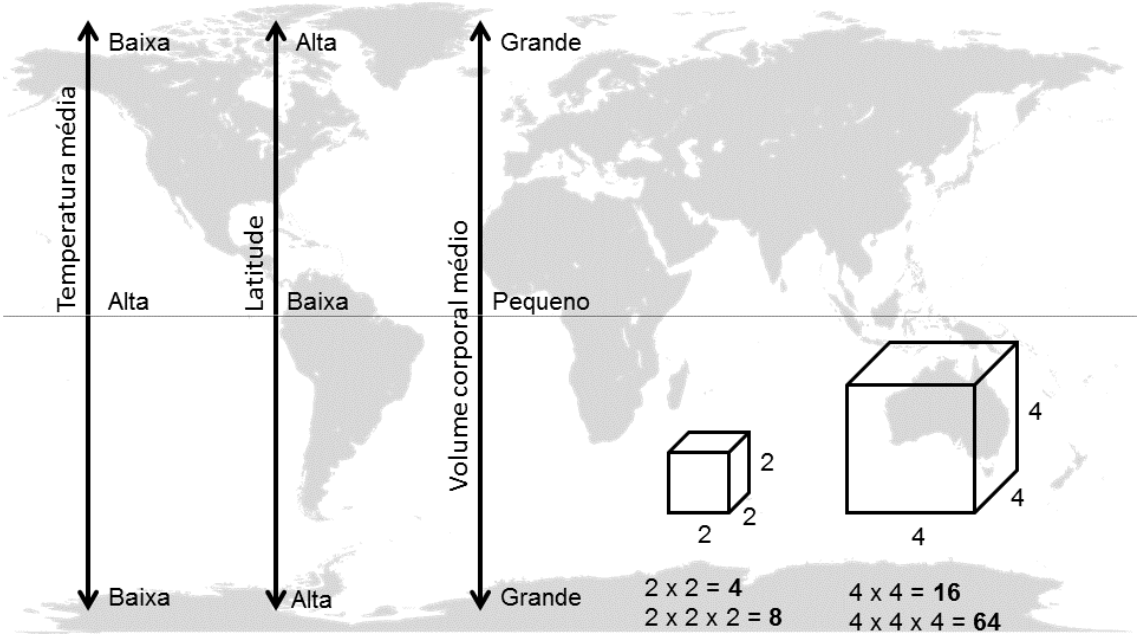

Figura 1: Representação esquemática da Regra de Bergmann (Bergmann, 1847), ilustrando a relação de volume corporal médio com temperatura e latitude. Os cubos demonstram que o aumento do volume de um determinado corpo implica em um aumento de área em menor proporção, otimizando a conservação de calor.

Originalmente, a existência de uma relação de temperatura ambiental com tamanho corporal foi postulada em 1847 por Carl Bergmann e, segundo a tradução do texto original em alemão realizada por Clauss *et al*. (2013), Bergmann disserta acerca de diferenças entre "organizações similares" (traduzido literalmente do referido artigo) baseadas em posições filogenéticas, o que é interpretado por Blackburn *et al*. (1999) como diferenças interespecíficas. Um estudo abrangente realizado por James (1970), considerando variações intraespecíficas, identificou correlações negativas de tamanho de asa com temperatura do ar em aves terrestres na América do Norte. Nesse contexto,

Blackburn *et al*. (1999) argumentam que o estudo conduzido por James (1970) tenha sido o primeiro a demonstrar amplamente a aplicação do conceito de Carl Bergmann em nível intraespecífico, sugerindo que a correlação de tamanho corporal com temperatura em nível intraespecífico seja denominada Regra de James, embora diversos estudos recentes permaneçam utilizando o termo Regra de Bergmann para evitar confusões (*e.g.* Fisher *et al*., 2010; Berke *et al*., 2013).

Independente do termo empregado e do gradiente climático utilizado para explicar a diversidade biológica, a ideia de que a estrutura populacional entre indivíduos de uma mesma espécie resulta das múltiplas pressões seletivas às quais os organismos estão expostos (Mayr, 1956) é amplamente aceita, e a identificação dessas variações é crucial para a compreensão dos mecanismos de divergência (Rensch, 1938). De fato, conhecer a diversidade intraespecífica e associá-la à heterogeneidade da paisagem pode fornecer um melhor entendimento sobre os promotores de isolamento populacional e sobre como os processos evolutivos podem ser utilizados para prever os impactos de rápidas mudanças climáticas sobre a biodiversidade (Orsini *et al*., 2013). Para organismos marinhos, as características da paisagem marinha também funcionam como barreira para o isolamento populacional como, por exemplo, temperatura superficial do mar para odontocetos (Amaral *et al*., 2012) ou batimetria para peixes (Hyde *et al*., 2008) e macroalgas - *kelps* (Johansson *et al*., 2015). No entanto, estudar as forças evolutivas agindo sobre o fluxo gênico em populações marinhas pode ser ainda mais complexo, em função da dificuldade de caracterizar a paisagem marinha e obter dados sobre demografia e relações tróficas (Selkoe *et al*., 2008).

Nos primeiros estudos desenvolvidos sobre isolamento populacional, foi sugerido que a diferenciação genética entre populações aumenta à medida que a distância

geográfica também aumenta (*e.g*. Wright, 1943). De acordo com o modelo de Isolamento por Distância, na ausência de seleção natural e taxas reduzidas de dispersão, a deriva genética seria responsável por aumentar a diferenciação populacional de maneira linear à distância geográfica entre populações, ou seja, quanto mais distantes geograficamente, maior a diferenciação genética entre duas populações (Figura 2; Wright, 1943; 1946). O modelo de Isolamento por Distância tem sido demonstrado para grandes escalas espaciais e aplicado para organismos com baixa capacidade de mobilidade e dispersão, resultando em um padrão de autocorrelação espacial na distribuição da variação genética (Meirmans, 2012) comumente aplicado a plantas (Vekemans & Hardy, 2004) e invertebrados sésseis (Maier *et al.*, 2005).

Figura 2: Representação esquemática do modelo de Isolamento por Distância proposto por Wright (1943), no qual a diferenciação genética entre duas populações aumenta à medida que a distância geográfica aumenta. Na figura, quanto maior a diferença de coloração, maior a distância genética. Fonte: modificado de http://www.afsc.noaa.gov/Quarterly/jfm2011/divrptsREFM7.htm

Alternativamente, tem sido demonstrado que não apenas a distância geográfica influencia a estruturação populacional, mas a adaptação dos organismos às características ambientais locais também pode reduzir o fluxo gênico e ser um importante fator promotor de diferenciação populacional, especialmente em vertebrados (Sexton *et al*., 2014). O

modelo de Isolamento pelo Ambiente pode ser definido "como um padrão no qual a diferenciação genética aumenta à medida que as diferenças ambientais aumentam, independentemente da distância geográfica entre duas populações" (Wang & Bradburd, 2014). Nesse contexto, a heterogeneidade da paisagem é um pressuposto básico do modelo de Isolamento pelo Ambiente e, além disso, alguns processos ecológicos são conhecidos por contribuir para o isolamento populacional, como dispersão assimétrica (Edelaar & Bolnick, 2012), seleção sexual, e seleção natural contra imigrantes, ou seja, o favorecimento de genótipos nativos por conta da adaptação às condições locais (Hendry, 2004). Portanto, variações ambientais de pequena escala são suficientes para funcionar como barreiras entre populações como, por exemplo, o tipo de hábitat para peixes recifais (Rocha *et al*., 2005), diferenças no tamanho de sementes usadas como alimento por Passeriformes (Ryan *et al*., 2007), tipo de floresta para orquídeas epífitas (Mallet *et al*., 2014), e topografia para gafanhotos (Noguerales *et al*., 2016).

Desde a última década, um emergente campo de estudo denominado "genética da paisagem" tem unido as ferramentas genéticas à ecologia espacial para auxiliar a compreender o papel da heterogeneidade ambiental sobre a distribuição da biodiversidade (Manel *et al*., 2003). Para isso, a diversidade genética acessada através de vários marcadores moleculares tem sido confrontada com as variáveis ambientais de onde indivíduos e populações ocorrem. Entre esses marcadores estão os microssatélites, os quais são compostos por sequências curtas (1 a 6 pares de base) que podem se repetir consecutivamente de 10 a 100 vezes (Tautz & Renz, 1984). As taxas de mutação de microssatélites são relativamente altas quando comparadas a outros marcadores moleculares amplamente utilizados (*e.g.* mtDNA e íntrons nucleares). As mutações nos microssatélites ocorrem através da geração de sequências imperfeitas por um mecanismo

chamado de derrapagem de replicação (ou "*replication slippage*"), causando a adição ou remoção de uma das sequências do microssatélite (Madesis *et al*., 2013). Isso significa dizer que as sequências de microssatélites podem variar entre espécies e até entre populações de uma mesma espécie, e que a informação fornecida pelas frequências de alelos de microssatélites viabilizam o estudo da genética de populações e de vários processos evolutivos, como seleção natural, deriva genética, mutação, fluxo gênico e sistemas de acasalamento (Kim & Sappington, 2013). Portanto, analisar variações intraespecíficas de frequências alélicas de marcadores polimórficos, como microssatélites, e interpretar os resultados à luz de gradientes e diferenças nas condições ambientais, permite compreender a forma como a ecologia interfere na evolução e vice-versa (Pelletier *et al*., 2009).

O estudo da genética da paisagem tem utilizado ferramentas estatísticas já solidamente estabelecidas, mas também tem influenciado o desenvolvimento de novas técnicas para analisar os dados gerados. Entre as técnicas mais amplamente aplicadas para comparar dados genéticos e ambientais estão: análises de correspondência de matrizes como o teste de Mantel, o qual permite comparar matrizes pareadas de distâncias genética, geográfica e de características ambientais através de diversos índices de similaridade ou dissimilaridade; a Análise de Redundância, uma técnica multivariada capaz de comparar duas matrizes quadradas com dados genéticos e ambientais, apontando correlações entre as variáveis de cada matriz e utilizando os dados de uma para explicar a variabilidade da outra; e modelos de regressão, os quais são rodados com dados ambientais como variáveis independentes para explicar a distância genética entre populações (Manel & Holderegger, 2013). Adicionalmente, outras ferramentas têm aperfeiçoado as interpretações da genética de paisagem, como o refinamento de sistemas de informação geográfica

(Etherington, 2011; Thomassen *et al*., 2011) e o avanço da genômica (Schwartz *et al*., 2010; Manel *et al*., 2012), tornando a genética de paisagem uma técnica promissora para explicar a distribuição espacial da biodiversidade.

Embora tenha completado apenas 10 anos em 2016, o campo da genética de paisagem marinha (ou "*seascape genetics*") também tem experimentado um importante aumento no número de publicações e no refinamento de técnicas analíticas (Selkoe *et al*., 2016). No entanto, os inerentes obstáculos para caracterizar o altamente dinâmico ambiente marinho, bem como a dificuldade em definir distribuição e área de vida dos organismos marinhos, tornam ainda mais desafiadora a tarefa de utilizar dados ambientais para explicar a conectividade de populações no oceano. Um interessante exemplo é o grupo das aves marinhas, as quais são altamente móveis, com capacidade de realizar as maiores migrações observadas no Reino Animal (Egevang *et al*., 2010; Fijn *et al*., 2013), ocupando vastas extensões do oceano com variados gradientes ambientais e dificultando a definição de área de vida. No entanto, a estratégia de reprodução em colônias e a elevada filopatria, tornam possível a definição de um desenho amostral baseado em tais agrupamentos reprodutivos para testar o efeito das características ambientais do entorno da colônia sobre a estrutura populacional.

Embora a utilização de dados ambientais empíricos seja escassa, alguns estudos sugerem influência do ambiente na promoção de adaptação local e isolamento populacional em aves marinhas. Por exemplo, fragatas ou tesourões *Fregata magnificens* são amplamente distribuídas nos Oceanos Atlântico e Pacífico, e apresentam a menor relação de volume corporal com área de asa ("*wing loading*") e também habilidade de identificar áreas de baixa pressão e utilizar ventos térmicos para movimentos migratórios, o que confere a essa espécie uma alta capacidade de deslocamento (Nelson, 2005;

Weimerskirch *et al*., 2016). No entanto, a população que se reproduz nas ilhas Galápagos está isolada geneticamente das populações das Américas Central e do Sul, o que tem sido atribuído à adaptação local às condições oceanográficas encontradas próximas às colônias (Hailer *et al*., 2010). A estruturação populacional do pinguim gentoo *Pygoscelis papua* também tem sido atribuída às características oceanográficas do entorno das colônias (de Dinechin *et al*., 2012) e, mais recentemente, foi sugerido que a Frente Polar haja como uma barreira de isolamento entre populações da espécie (Levy *et al*., 2016) . Desse modo, a estruturação populacional de organismos altamente móveis entre colônias geograficamente próximas sugere que as características ambientais às quais as aves estão expostas tenham papel primário na seleção de fenótipos ótimos, influenciando nas taxas de dispersão do grupo (revisado por Friesen *et al*., 2007; Friesen, 2015).

Algumas ferramentas adicionais têm auxiliado na compreensão das relações das aves marinhas com o ambiente. A telemetria, a qual pode ser definida como um conjunto de técnicas que utilizam equipamentos eletrônicos para rastrear diversos comportamentos de organismos de vida livre, tem se tornado indispensável em estudos que buscam entender uso do hábitat e comportamento de forrageio, uma vez que esses organismos passam grande parte do seu ciclo de vida fora do alcance direto do observador (Ropert-Coudert & Wilson, 2005; Candia-Gallardo *et al*., 2010). Na prática, tratam-se de equipamentos fixados diretamente no corpo do animal com capacidade para armazenar, ou mesmo enviar ao observador em tempo real, dados de localização geográfica, comportamentais e/ou das variáveis ambientais às quais o aparelho está exposto (Wilson *et al*., 2002). Para isso, os aparelhos de rastreamento remoto têm sofrido um processo de miniaturização desde o primeiro gravador de profundidade fixado em uma foca de Weddell em 1965 (Kooyman, 1965), simultaneamente a uma redução de custo, aumento

de vida da bateria e da disponibilidade de modelos no mercado (Wilson & Vandenabeele, 2012).

Através dessas tecnologias, foi possível descobrir, por exemplo, que populações adjacentes de *Morus bassanus* podem apresentar segregação de áreas de alimentação no mar (Figura 3; Wakefield *et al*., 2013), que os trinta-réis-do-Ártico *Sterna paradisaea* alimentam-se na Antártica durante o período não-reprodutivo e, para isso, realizam a maior migração animal já registrada (Fijn *et al*., 2013), e que o pinguim-imperador *Aptenodytes forsteri* pode mergulhar a mais de 500 m de profundidade (Wienecke *et al*., 2007). O estudo de diferenças intraespecíficas também tem se beneficiado com a telemetria. As aves marinhas são caracterizadas por um marcado dimorfismo sexual de tamanho, o que tem sido atribuído a uma estratégia de partição de nicho, seleção sexual, ou a diferentes papéis parentais durante a reprodução (Serrano-Meneses & Székely, 2006). Nesse contexto, a utilização de rastreadores remotos tem demonstrado extensivamente a utilização diferencial de áreas no mar por machos e fêmeas de uma mesma população (*e.g.* Jaeger *et al*., 2014, Hennicke *et al*., 2015) e até mesmo diferenças entre indivíduos do mesmo sexo (Sommerfeld *et al*., 2013), esclarecendo a função de características biológicas básicas, como a variação no tamanho corporal ou diferenças na personalidade, a qual é definida como o conjunto de características comportamentais intrínsecas dos indivíduos (Patrick & Weimerskirch, 2014).

O estudo da dieta é outra maneira de compreender a forma como as aves marinhas interagem com o ambiente. Em geral, as interações de aves marinhas com suas presas tendem a ser controladas de baixo para cima, ou seja, a disponibilidade de presas é que interfere na abundância e distribuição das aves (Grémillet & Boulinier, 2009; Chambers *et al*., 2011), embora existam exemplos do contrário (Gaston *et al*., 2007; Oppel *et al*.,

2015). Tradicionalmente, o estudo da dieta é realizado através da observação direta em campo ou da quantificação dos itens encontrados em conteúdos estomacais obtidos por necropsia, lavagem estomacal, ou regurgitados espontâneos, os quais são analisados através de índices de importância relativa que consideram, entre outras informações, frequência de ocorrência, volume, e abundância dos itens (Costello, 1990; Brown *et al*., 2012). Embora tais técnicas tenham a capacidade de identificar de uma maneira direta as interações presa-predador, possuem uma forte limitação temporal, visto que possibilitam conhecer apenas a última, ou últimas, ingestões pelo animal. Portanto, é imprescindível que ferramentas complementares preencham as lacunas das técnicas de estudo direto da dieta, tornando os resultados mais robustos e refinando o conhecimento sobre as interações presa-predador em uma maior janela espacial e temporal.

Nesse contexto, a análise de isótopos estáveis tem sido utilizada como forma de reconstruir a dieta de organismos de vida livre e estimar a partilha de recursos entre indivíduos ou grupos (Boecklen *et al*., 2011). Os isótopos estáveis são formas do mesmo elemento que diferem no número de nêutrons no núcleo, e fornecem uma forma natural de rastrear a ciclagem dos elementos (Fry 2006). Na prática, a medição de isótopos estáveis indica a proporção entre isótopos leves (*e.g.* ^{12}C, ^{14}N) e pesados (*e.g.* ^{13}C, ^{15}N), o que, por sua vez, é o resultado da assimilação dos componentes do alimento, como proteínas, lipídios e carboidratos (Hobson 1999). Embora seja uma técnica indireta para estudo da ecologia trófica, a análise de isótopos estáveis não apenas complementa os métodos tradicionais de estudo da dieta, mas também oferece algumas vantagens importantes, como a utilização de amostras obtidas por métodos pouco invasivos, a estimativa de interações tróficas em variadas escalas espaciais e temporais, e a identificação de relações presa-predador em lugares fora do alcance de observadores (Fry,

2006). As medições de isótopos estáveis têm se tornado importantes para compreender as estruturas tróficas de uma comunidade e, para isso, razões isotópicas de carbono ($^{13}C/^{12}C$) e nitrogênio ($^{15}N/^{14}N$) têm sido amplamente utilizadas (*e.g.* Kelly, 2000; Perkins *et al*., 2014), embora o estudo de interações tróficas a partir de outros elementos, como enxofre (Connolly *et al*., 2004; Mittermayr *et al*., 2014), hidrogênio e oxigênio (Pekarsky *et al*., 2015; Zanden *et al*., 2016), tenha expandido nos últimos anos.

As medições das razões isotópicas de carbono e nitrogênio, em comparação com um padrão, fornecem importantes informações a partir da interpretação de cada elemento separadamente e também dos elementos juntos. A razão isotópica de nitrogênio (ou $\delta^{15}N$) tende a ser maior (*i.e*. maior proporção de isótopos pesados em relação ao padrão de referência) de um nível trófico para outro em uma proporção de 3,4‰ (Post *et al*., 2002), embora esse valor possa variar entre 2,5‰ e 5‰ de acordo com a proporção de proteína na dieta (DeNiro & Epstein, 1981; Hobson & Clark, 1992; Bearhop *et al*., 2002). Com isso, a medição de isótopos estáveis de nitrogênio pode ser utilizada para a determinação da posição trófica de um organismo, o que representa uma valiosa informação para a compreensão da estrutura da teia trófica de uma comunidade (Post, 2002; Vanderklift & Ponsard, 2003; Caut *et al*., 2009).

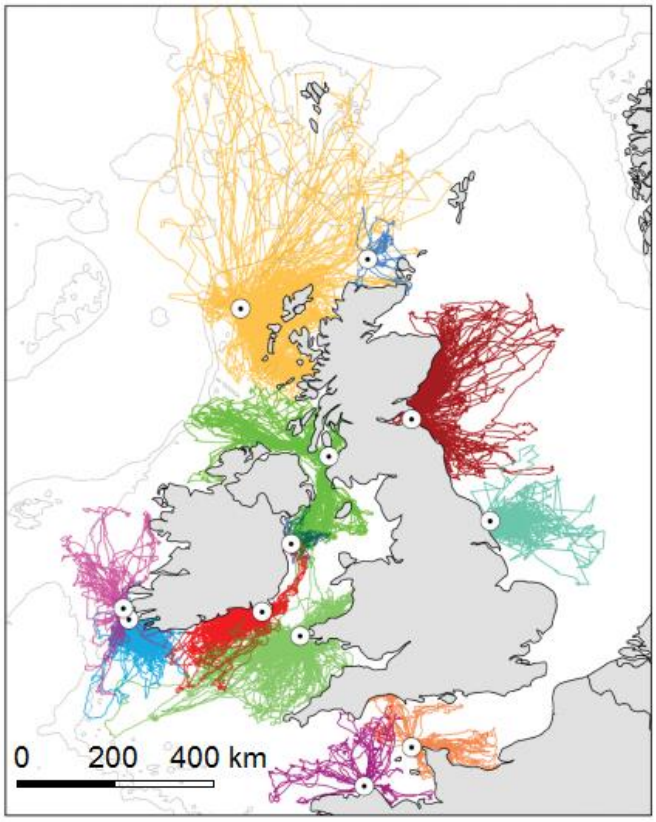

Figura 3: Representação da segregação de áreas de alimentação entre diferentes colônias de *Morus bassanus* no entorno do Reino Unido. A figura é uma compilação de dados de distribuição espacial, obtidos com equipamentos eletrônicos de rastreamento remoto fixados às aves, durante viagens de alimentação em período reprodutivo. Cada cor representa uma colônia distinta. Fonte: Modificado de Wakefield *et al*. (2013).

Por outro lado, a discriminação trófica da razão isotópica de carbono (ou $\delta^{13}C$) entre a fonte e o consumidor normalmente varia de -0,7% a 1,9%, o que restringe a utilização de valores de $\delta^{13}C$ para estimar níveis tróficos de uma comunidade, visto que a variação de um nível trófico para outro é limitada (Post, 2002; Caut *et al*., 2009; Perkins *et al*., 2014). No entanto, os valores de $\delta^{13}C$ variam de acordo com as características dos produtores primários de uma comunidade e, portanto, são úteis para identificar as vias energéticas em sistemas com diferentes fontes basais (Post, 2002; Perkins *et al*., 2014).

Por exemplo, em ambientes aquáticos o perifíton tende a ser enriquecido em ^{13}C, quando comparado ao fitoplâncton, devido à menor turbulência à qual está exposto (France, 1995). Isso resulta em maiores valores de $\delta^{13}C$ em ambientes litorâneos em comparação aos ambientes pelágicos, o que confere ao carbono uma relevante utilidade para estimar a distribuição espacial dos organismos aquáticos (France, 1995; veja Mancini & Bugoni, 2014, para uma representação esquemática das variações de razões isotópicas de carbono e nitrogênio no ambiente marinho). Técnicas estatísticas univariadas e multivariadas possibilitam a comparação de médias e variâncias considerando os valores de razão isotópica de carbono e nitrogênio para grupos pré-determinados. Além disso, abordagens Bayesianas permitem estimar a amplitude do nicho isotópico bidimensional de um determinado grupo de organismos e a contribuição de presas potenciais para a dieta de um predador a partir de valores de carbono e nitrogênio (Parnell *et al*., 2010; Jackson *et al*., 2011).

Portanto, atualmente há uma ampla gama de ferramentas analíticas disponíveis para o estudo das interações ecológicas e suas relações com os processos evolutivos em populações naturais. Além disso, a obtenção remota de dados ambientais de regiões de difícil acesso, como a plataforma continental e o ambiente pelágico, através de imagens de satélite e boias oceanográficas, permitiu um importante avanço no conhecimento sobre as características e dinâmica da paisagem marinha, viabilizando estudos que busquem padrões de distribuição da biodiversidade relacionados às variáveis ambientais.

A linha de costa brasileira, por apresentar quase 7400 km de extensão, possui uma considerável heterogeneidade de ambientes que vai desde a região de clima estritamente tropical com águas costeiras influenciadas pela Corrente Norte do Brasil, a mais de 4°N, até a região de clima subtropical influenciada pela Confluência Brasil-Malvinas, a

aproximadamente 33°S (Seeliger & Kjerfve, 2001). Entre esses dois pontos, ocorrem ambientes dinâmicos que sofrem influência das correntes oceânicas e também de fatores locais da plataforma continental, como desembocaduras de rios e baías, e sistemas de mistura da coluna d'água gerados por ventos, pela topografia de fundo ou pelo encontro de águas de plataforma, como vórtices, ressurgências de pequena escala, e frentes oceânicas (Kjerfve *et al*., 2001; Valentin, 2001; Möller-Jr *et al.*, 2008; Piola *et al*., 2008). Portanto, a costa brasileira é composta por grande parte dos ecossistemas marinhos encontrados no sudoeste do Oceano Atlântico, região que também é composta por ilhas oceânicas fora da plataforma continental, como o Arquipélago de Fernando de Noronha e o Atol das Rocas. Por conta da heterogeneidade de características físicas, essa região torna-se uma interessante área de estudo para avaliar o efeito do ambiente sobre a distribuição da diversidade biológica.

Nesse contexto, as aves marinhas surgem como interessantes modelos de estudo, visto que estão amplamente distribuídas em todos os oceanos, tornando possível comparar populações ocorrendo ao longo de gradientes ambientais (Schreiber & Burger, 2001). Esse é o caso do atobá-marrom (*Sula leucogaster*), uma ave estritamente marinha, com dimorfismo sexual reverso, ou seja, fêmeas com maior tamanho corporal em relação aos machos, que habita latitudes tropicais e subtropicais em todos os oceanos (Figura 4; Carboneras, 1992). Atobás-marrons, bem como os demais representantes da família Sulidae, possuem um comportamento de forrageio peculiar, lançando-se em queda livre para atingir velocidades que podem chegar a 100 km/h antes do mergulho (em inglês, "*plunge diving*") (Nelson, 2005). Durante o período reprodutivo, apresentam cuidado biparental alimentando-se no entorno das colônias e, durante o período não-reprodutivo,

não realizam movimentos previsíveis de migração, permanecendo próximos às colônias (Nelson, 2005).

A diversidade genética intraespecífica é alta em atobás-marrons, e distintas populações foram reconhecidas nos Oceanos Pacífico, Índico e Atlântico, sendo que, apenas no último, três populações foram identificadas (Ilhas Ascensão, Arquipélago de Cabo Verde, e Pequenas Antilhas) (Morris-Pocock *et al*., 2011). No entanto, as colônias de atobá-marrom localizadas no sudoeste do Oceano Atlântico não foram consideradas no estudo supracitado, apesar de ocorrerem desde o Arquipélago de Moleques do Sul, a 27° de latitude sul, até o Arquipélago de São Pedro e São Paulo (ASPSP), sobre a linha do Equador (Sick, 1997). Entre esses dois extremos de distribuição, os atobás-marrons nidificam em ilhas na costa sudeste brasileira, como Laje de Santos e Ilhas Cagarras, no Arquipélago de Abrolhos, além do Arquipélago de Fernando de Noronha e no Atol das Rocas (Branco, 2004). Portanto, a espécie está exposta ao gradiente de condições ambientais desde a região nerítica subtropical, ao sul, até as zonas pelágicas tropicais, ao norte, representando um potencial organismo-modelo para avaliação do papel do ambiente na moldagem de fenótipos e no fluxo gênico entre colônias.

Figura 4: Casal de atobás-marrons, *Sula leucogaster*, no Arquipélago de São Pedro e São Paulo. Além do maior tamanho corporal em relação aos machos, fêmeas (direita) possuem pele facial amarelo-clara com uma mancha preta em frente ao olho, enquanto machos possuem um gradiente que vai do amarelo na base do bico até uma ponta pálida, com um anel azul no entorno dos olhos (esquerda) (Nelson, 2005).

No extremo sul da distribuição da espécie no Oceano Atlântico estão as Ilhas Moleques do Sul, as quais são sazonalmente influenciadas pela Frente Subtropical de Plataforma (Piola *et al*., 2008), elemento chave para a elevada produtividade local (Odebrecht & Castello, 2001; Figura 5). Moleques do Sul abriga uma colônia de, aproximadamente, 1700 indivíduos (Branco *et al*., 2010), os quais utilizam preferencialmente as encostas íngremes das ilhas para nidificação (Branco *et al*., 2013) e beneficiam-se de descartes da pesca de camarão, especialmente de espécies demersais e bentônicas (Branco *et al*., 2005). Na plataforma continental da região sudeste brasileira

encontram-se as Ilhas Cagarras, as quais abrigam uma colônia de aproximadamente 2500 atobás-marrons, os quais nidificam tanto nas áreas íngremes no entorno das ilhas, como no topo levemente plano (Cunha *et al*., 2013; observação pessoal). As Ilhas Cagarras estão a pouco mais de 4 km da costa e são influenciadas pela desembocadura da altamente eutrofizada Baía de Guanabara (Kjerfve *et al*., 2001) e pelo sistema de ressurgência de Cabo Frio, o qual é um processo ascendente, regido pelo vento, da Água Central do Atlântico Sul (Valentin, 2001). Ainda sobre a plataforma continental e mais ao norte, está a colônia de atobás-marrons do Arquipélago de Abrolhos, a qual é composta por cerca de 550 indivíduos (Mancini *et al*., 2016), os quais utilizam, preferencialmente, bordas de escarpas para nidificação (Alves *et al*., 2000). Os atobás-marrons de Abrolhos, por sua vez, forrageiam na região influenciada pelo Rio Caravelas (Pereira, 2012), uma área rica em nutrientes que suporta o segundo maior manguezal da região nordeste brasileira (Herz, 1991).

Figura 5. Representação esquemática de localização e direção das principais massas de água e processos oceanográficos que influenciam a costa brasileira. As setas em cinza indicam correntes fora da plataforma continental, enquanto que as setas em preto indicam massas d'água e processos ocorrendo sobre a plataforma continental. Rocas = Atol das Rocas; FN = Arquipélago de Fernando de Noronha; ASPSP = Arquipélago de São Pedro e São Paulo.

Adicionalmente, o Arquipélago de Fernando de Noronha e o Atol das Rocas abrigam colônias de 190 e 220 atobás-marrons, respectivamente (Mancini *et al*., 2016). O entorno de ambos os sítios reprodutivos, os quais são separados por apenas 150 km, é influenciado primariamente pela Corrente Sul Equatorial, a qual é composta por águas quentes e oligotróficas, de modo que os picos de produtividade primária observados na região estão associados essencialmente à interação das massas d'água com montes submarinos, causando uma distribuição em mancha dos nutrientes (Souza *et al*., 2013). No Atol das Rocas, os atobás-marrons nidificam em áreas periféricas associadas à vegetação (Kohlrausch, 2003), enquanto que em Fernando de Noronha, utilizam áreas rochosas nas bordas das ilhas secundárias (Schulz-Neto, 2004).

No Arquipélago de São Pedro e São Paulo, o qual é o arquipélago mais setentrional sob jurisdição brasileira e também parte de uma fratura da Dorsal Mesoatlântica, está uma colônia de atobás-marrons com aproximadamente 600 indivíduos (Mancini *et al*., 2016). O arquipélago apresenta características físicas peculiares, como uma área emersa total de apenas 0,01 km^2 e dinâmica oceanográfica similar à de montes submarinos, sendo influenciado pela Corrente Sul Equatorial, a qual flui para oeste (Richardson & Walsh, 1986), e pela Contracorrente Equatorial, a qual flui em sentido oposto com o núcleo a aproximadamente 80 m de profundidade (Veleda *et al*., 2012). Quando atinge o relevo submarino de São Pedro e São Paulo, a Contracorrente Equatorial diminui sua velocidade, gerando vórtices e ressurgência de pequena escala a leste, e aumentando o tempo de residência de seus nutrientes nas águas do entorno do arquipélago (Araujo & Cintra, 2009; Soares *et al*., 2012). Devido à pequena área útil para nidificação, a colônia de atobás-marrons apresenta alta densidade, com distância média entre ninhos de 1 m e intensa disputa por espaço, especialmente entre fêmeas, as quais

são as principais responsáveis pela conquista e defesa de territórios para nidificação (Kohlrausch, 2003). Além disso, a paisagem da colônia é bastante heterogênea, o que faz com que haja diferença na qualidade dos lugares disponíveis para nidificação, variando de lugares onde os ninhos são frequentemente destruídos por marés de sizígia, até lugares altos e protegidos das ondas (observação pessoal). No entanto, a oferta de alimento, essencialmente peixes-voadores (Both & Freitas, 2001), parece ser abundante, visto que a atividade reprodutiva é constante e intensa no arquipélago, com baixa ocorrência de ninhos inativos e sem picos ao longo do ano (Kohlrausch, 2003; Barbosa-Filho & Vooren, 2010).

Em resumo, os atobás-marrons nidificam em colônias com marcantes diferenças ambientais ao longo do Oceano Atlântico sudoeste, e parecem ser fortemente dependentes das condições locais de cada arquipélago, visto que não realizam movimentos migratórios previsíveis durante o período não-reprodutivo e há poucos registros de deslocamento entre colônias baseados em recuperação de anilhas metálicas para marcação individual (Efe *et al*., 2006). Além disso, diferenças entre as referidas colônias de atobás-marrons já foram observadas em relação à amplitude do nicho trófico baseado em dados isotópicos de carbono e nitrogênio (Mancini *et al*., 2014), e aos haplótipos da região controladora do DNA mitocondrial (Baumgarten, 2003). Portanto, as características locais de cada colônia poderiam estar selecionando os caracteres relacionados ao *fitness* e ajustando os fenótipos de acordo com as pressões seletivas de cada sítio reprodutivo, o que levaria, em última instância, à diminuição do fluxo gênico entre as colônias com as maiores diferenças ambientais a partir da seleção contra imigrantes (Hendry, 2004).

Nesse contexto, as variabilidades fenotípica e genotípica de atobás-marrons reproduzindo nas colônias do sudoeste do Oceano Atlântico foram analisadas e

confrontadas com dados ambientais, a fim de compreender o papel do ambiente para a estruturação populacional, o que foi conduzido através dos objetivos detalhados a seguir.

OBJETIVOS

Objetivo geral

Avaliar o papel das variáveis ambientais e características intrínsecas de cada colônia como pressões de seleção sobre a diversidade de atobás-marrons na região sudoeste do Oceano Atlântico.

Objetivos específicos

1. Identificar a variabilidade fenotípica de atobás-marrons entre as colônias do Oceano Atlântico sudoeste;

2. Determinar a associação do padrão de distribuição espacial da variabilidade fenotípica com variáveis ambientais, como temperatura do ar (Regra de Bergmann), temperatura superficial do mar, e concentração de clorofila α;

3. Determinar a estruturação populacional atual de atobás-marrons entre as colônias do Oceano Atlântico sudoeste;

4. Estimar a influência de variáveis ambientais, relações tróficas, e parâmetros demográficos de cada colônia sobre a estruturação populacional de atobás-marrons;

5. Avaliar o papel da dieta, comportamento de forrageio, e qualidade do ninho como pressões de seleção sobre os fenótipos de atobás-marrons do Arquipélago de São Pedro e São Paulo.

HIPÓTESES

1. Em função da ampla variação latitudinal considerada (0−27°S) e, consequentemente, do marcante gradiente de temperatura do ar, atobás-marrons apresentarão forte diferenciação populacional fenotípica, com maiores indivíduos ocorrendo em maiores latitudes, consistentemente com a Regra de Bergmann;

2. Como sugerido para aves marinhas, diferenças na paisagem marinha entre colônias levam à diferenciação populacional a partir de adaptação local. Portanto, a diferenciação genotípica e taxa de dispersão entre colônias será proporcional às diferenças ambientais observadas, demonstrando que o modelo de Isolamento pelo Ambiente explica melhor a diversidade de atobás-marrons em comparação ao modelo de Isolamento por Distância;

3. Por conta das peculiaridades do Arquipélago de São Pedro e São Paulo, como o isolamento geográfico, a pequena área emersa, e a abundante oferta de alimento, os atobás-marrons reproduzindo naquela colônia estarão isolados geneticamente e apresentarão diferenças fenotípicas intrapopulacionais relacionadas à dieta, comportamento de forrageio, e qualidade do ninho.

MATERIAL E MÉTODOS

Amostragens

O presente estudo foi dividido em três partes, as quais são apresentadas em forma de anexo ao final da tese, e portanto, esta seção é uma compilação dos materiais e métodos utilizados nos três anexos da tese. O Anexo 1 envolveu a coleta de dados morfométricos de atobás marrons e obtenção de dados físicos para todas as colônias estudadas. O Anexo 2 envolveu coleta de amostras biológicas para análises molecular e de isótopos estáveis, além da utilização dos dados físicos obtidos para o Anexo 1 e de dados demográficos presentes na literatura. Por fim, o Anexo 3 envolveu amostragem massiva dos atobás-marrons no ASPSP, a partir da coleta de dados morfométricos, rastreamento remoto de atividades de forrageio, coleta de material biológico para análises da dieta, além da medição de características dos ninhos.

Área de estudo

O presente estudo foi conduzido em seis colônias de atobás-marrons distribuídas ao longo de um gradiente latitudinal que vai de 0° (ASPSP) até 27°S (Moleques do Sul), no sudoeste do Oceano Atlântico (Figura 1 − Anexo 1). As colônias apresentam distâncias variáveis a partir da costa, de ~4 km (Ilhas Cagarras) até ~1100 km (ASPSP), e também distintas abundâncias de atobás-marrons, variando de ~140 (Atol das Rocas) a ~2500 indivíduos (Ilhas Cagarras) (Tabela 1 − Anexo 1). Moleques do Sul, Cagarras, e Abrolhos estão localizados sobre a plataforma continental, enquanto que Fernando de Noronha,

Atol das Rocas e ASPSP possuem origem vulcânica e estão fora da plataforma continental. Este último é parte de uma fratura da Dorsal Mesoatlântica. Distâncias entre arquipélagos variam de ~150 km, entre Atol das Rocas e Fernando de Noronha, até ~3900 km, entre ASPSP e Moleques do Sul (Tabela 2 − Anexo 2). Todos os seis arquipélagos são Unidades de Conservação, mas apenas em Cagarras e Moleques do Sul não há presença humana permanente.

As localidades de estudo apresentam diferenças em relação à dinâmica oceanográfica do entorno. As colônias costeiras (Moleques do Sul, Cagarras, e Abrolhos) são influenciadas, principalmente, pelas águas de plataforma, como a Água Tropical, a qual flui para sul e é um ramo costeiro da Corrente do Brasil (Möller-Jr *et al*., 2008). Durante o inverno, as ilhas Moleques do Sul também são influenciadas pelas águas frias e menos salinas, mas ricas em nutrientes, oriundas da descarga do Rio da Prata (Piola *et al*., 2008). Por outro lado, Fernando de Noronha, Atol das Rocas, e ASPSP são influenciados primariamente pela Corrente Sul Equatorial, a qual flui para oeste, sendo que o último também é influenciado pela Subcorrente Equatorial, a qual flui no sentido oposto com o núcleo a aproximadamente 80 m de profundidade (Richardson & Walsh, 1986; Veleda *et al*., 2012). A Subcorrente Equatorial diminui sua velocidade quando atinge o ASPSP, gerando vórtices e ressurgência de pequena escala no lado leste do arquipélago e aumentando o tempo de residência dos nutrientes na região, uma dinâmica similar àquela observada em montes submarinos (Mullineaux & Mills, 1997; Araujo & Cintra, 2009; Soares *et al*., 2012).

Além da peculiar dinâmica oceanográfica e grande distância da costa, o ASPSP é composto por 10 ilhotas que totalizam uma área de 0,01 km^2. O arquipélago abriga colônias do trinta-réis-escuro, *Anous stolidus*, e do trinta-réis-preto, *A. minutus*, além do

atobá-marrom, o qual nidifica principalmente na Ilha Belmonte (Mancini *et al*., 2016). A colônia de atobás-marrons da Ilha Belmonte, a qual tem 21 m de altitude e 0,006 km^2 de área, é composta por ~150 ninhos (este estudo; Kohlrausch, 2003), os quais estão distribuídos nas rochas íngremes da face noroeste da ilha (Figura 1 − Anexo 3). A colônia é heterogênea em relação às características da paisagem, de modo que os ninhos variam em altitude, distância do mar, suscetibilidade às ondas, e em distância para os ninhos adjacentes. Pelo fato do ASPSP estar localizado na linha do Equador, é diretamente influenciado pela sazonalidade da Zona de Convergência Intertropical, uma região de baixa pressão formada pelo encontro dos ventos originados a 30° de latitude em ambos os hemisférios, formando uma banda de nuvens que geram altas taxas de precipitação (Riehl, 1979). De fevereiro a maio, a Zona de Convergência Intertropical está entre 7°S e 8°N e, portanto, sobre o ASPSP, enquanto que em meados de abril a precipitação máxima acumulada atinge 370 mm no arquipélago (Soares *et al*., 2012).

Coleta de dados morfométricos

De 2010 a 2015, atobás-marrons adultos foram capturados em seus ninhos manualmente ou com o auxílio de puçá, e foram obtidas as seguintes medidas corporais de cada indivíduo: comprimento do cúlmen (cúlmen exposto); comprimento do tarso (desde o meio da articulação meiotarsal até a extremidade distal do tarsometatarso); corda da asa não achatada (desde a articulação do carpo até a ponta da rêmige primária mais longa); e massa corporal. A corda da asa foi medida com régua metálica com precisão de 1 mm, enquanto que a massa corporal foi pesada com balança Pesola® com 10 g de precisão, e as demais medidas foram tomadas com paquímetros com 0,1 mm de precisão.

Adultos foram identificados pela coloração da plumagem, e o sexo dos adultos foi identificado pela coloração do bico e do entorno dos olhos (Nelson, 2005). Após a amostragem, cada atobá recebeu uma anilha metálica numerada individualmente, para evitar reamostragem, e foi liberado no mesmo local onde foi capturado.

Obtenção de dados ambientais

Dados de temperatura do ar e temperatura superficial do mar para o ASPSP, Fernando de Noronha e Atol das Rocas, foram obtidos das boias oceanográficas do Programa PIRATA (*Prediction and Research Moored Array in the Tropical Atlantic*) mais próximas de cada arquipélago, as quais estão localizadas a 0°N e 35°O para o ASPSP, e 8°S e 30°O para Fernando de Noronha e Atol das Rocas. Os mesmos dados para os arquipélagos costeiros foram obtidos a partir das boias fixas do Programa Nacional de Boias mais próximas de cada arquipélago: boia de Santa Catarina para Moleques do Sul (28,52°S e 47,37°O), boia de Guanabara para Cagarras (22,91°S e 43,15°O), e boia de Porto Seguro para Abrolhos (15,99°S e 37,95°O). A partir das boias PIRATA, foram extraídos dados médios de temperatura entre 1 de janeiro de 2000 e 1 de janeiro de 2015, e das boias fixas costeiras foram obtidos dados desde a data de lançamento de cada boia (março de 2011, abril de 2012, e julho de 2012, para Moleques do Sul, Cagarras, e Abrolhos, respectivamente) até março de 2015. Dados de concentração média de clorofila α, para verões entre 2002 e 2014, foram obtidos a partir do espectroradiômetro MODIS, o qual está a bordo do satélite Aqua da Agência Espacial Norteamericana NASA, utilizando o algoritmo Ocean Color Index. Foi obtida uma série de dados com resolução de 4 km/pixel e, a partir disso, foi calculado um valor médio de

concentração de clorofila α dentro de um raio de 40 km no entorno de cada colônia, baseado em estudos prévios que descreveram a distância máxima a partir da colônia que atobás-marrons utilizam durante viagens de alimentação (Weimerskirch *et al*., 2009; Soanes *et al*., 2015). Neste estudo, a concentração de clorofila α foi utilizada como um *proxy* de produtividade primária (Huot *et al*., 2007).

Coleta de material biológico

Amostras de sangue de atobás-marrons foram obtidas para análises moleculares e de isótopos estáveis, a partir da punção da veia tarsal com seringas e agulhas estéreis. Para as análise moleculares, ~200 µl foram coletados e armazenados em microtubos de 2 ml contendo álcool 70º, para posterior extração de DNA e amplificação das regiões de interesse em laboratório. Para as análises de isótopos estáveis, 1 ml de soro sanguíneo foi isolado a partir da coleta de 3 ml de sangue total, o qual foi armazenado em tubos não heparinizados de 3 ml e mantido em posição vertical à temperatura ambiente durante 10 a 30 min para coagulação, antes de ser centrifugado por 25 min a uma velocidade de 3000 RPM. O soro sanguíneo tem meia vida isotópica < 5 dias em aves (Boecklen *et al*., 2011) e, portanto, foi utilizado no estudo conduzido no Anexo 3 para representar o período imediatamente anterior ao rastreamento remoto das viagens de forrageio do indivíduo.

Além das amostras de sangue, ainda foram coletados regurgitados dos atobás-marrons do ASPSP para o estudo do Anexo 3. A regurgitação espontânea do conteúdo estomacal é um comportamento comum nas espécies da família Sulidae quando em situação de estresse (Nelson, 2005). Portanto, o material regurgitado dos atobás-marrons do ASPSP foi coletado durante o manuseio, identificado em nível de espécie, e medido o

comprimento total para os cefalópodes, e o comprimento da extremidade distal da cabeça até a forquilha da cauda para peixes, com régua metálica com precisão de 1 mm. Após isso, amostras de músculo de ~1 cm^3 dos itens identificados e medidos foram coletadas e armazenadas em microtubos contendo etanol absoluto (Bugoni *et al*., 2008), para servir como fonte nos modelos de mistura após medição de razões isotópicas de carbono e nitrogênio.

Extração de DNA e amplificação dos microssatélites

A extração do DNA foi realizada com um protocolo que utiliza NaCl a 5M (Medrano *et al*., 1990). A partir do DNA genômico total, foram amplificados nove *loci* de microssatélites com *primers* previamente descritos por Taylor *et al*. (2010). A cauda M13(-21) foi incorporada na extremidade 5' de cada *primer forward* (conforme Schuelke, 2000) e, consequentemente, aos produtos da Reação em Cadeia da Polimerase (PCR). As reações foram realizadas com volume final de 20 µl, contendo 20 a 30 ng de DNA, 10 pmol do *primer forward*, 10 pmol do *primer reverse*, 10 pmol de fluorescência HEX ou FAM, 10 mM de cada dNTP, 1,5 mM de $MgCl_2$, 1× de tampão de PCR, e 1 unidade de *Taq* DNA-Polimerase (Ludwig Biotec). O programa de PCR em *touchdown* proposto por Taylor *et al*. (2010) foi aplicado apenas para o *locus* Sv2B-138 e, para os demais, um novo programa foi desenvolvido em laboratório, como segue: 94°C por 5 min, seguidos por 35 ciclos de 94°C por 30 s, temperatura de anelamento por 30 s, e 72°C por 30 s, com alongamento final a 72°C por 10 min. A temperatura de anelamento foi ajustada para cada *locus*: 50°C para Sv2A-95, Sv2A-47 e Sv2B-27; 52°C para Sv2A-2 e Sn2A-123; 54°C para Sn2B-100; e 56°C para Sv2A-26 e Sn2B-83. Após isolamento dos produtos de PCR,

10 µl com concentração média de 100 ng/µl foram genotipados no analisador capilar ABI3730XL (Applied Biosystems), utilizando o marcador interno de tamanho 400 HD. Para calibração entre as rodadas de genotipagem, 5−10% de amostras já genotipadas foram enviadas em cada genotipagem subsequente.

Processamento de amostras para análise de isótopos estáveis

Amostras de músculo dos itens regurgitados foram lavadas em um extrator Soxhlet com condensador durante três sessões de 8 h cada, utilizando como solvente uma solução de clorofórmio e metanol em proporção de 2:1, respectivamente, a fim de remover lipídios (Logan & Lutcavage, 2008). A partir disso, amostras de músculo e de soro foram liofilizadas e homogeneizadas, e subamostras de 0,7 mg foram transferidas para cápsulas de estanho e enviadas para análise em espectrômetro de massa de razão isotópica com precisão de 0,2‰ tanto para carbono como para nitrogênio, no Stable Isotope Core, Washington State University (EUA). A diferença entre a razão da amostra e os padrões de referência internacional (Viena Pee Dee Belemnite para carbono e ar atmosférico para nitrogênio) foram expressadas em notação δ (‰) neste estudo, de acordo com a equação proposta por Bond & Hobson (2012):

$$\delta^{13}\text{C ou } \delta^{15}\text{N (‰)} = \left(\frac{R_{amostra}}{R_{referência}}\right) - 1 \text{ ,}$$

onde R corresponde à razão entre isótopos pesados e leves.

Esquema de amostragem dos atobás-marrons do ASPSP

Durante junho de 2013, maio/junho de 2014, e junho/julho de 2015, dados morfométricos e de massa corporal foram obtidos de atobás-marrons reproduzindo no ASPSP. Durante a expedição realizada em 2015, os atobás-marrons foram divididos em subgrupos baseados em tamanho corporal e, para isso, cada sexo foi tratado como um grupo distinto, em função do marcante dimorfismo sexual observado na espécie (Nelson, 2005). Para gerar um índice de tamanho corporal, a corda da asa, comprimentos do cúlmen e do tarso, e massa corporal, foram padronizados (subtraindo a média e dividindo pelo desvio padrão) e convertidos em componentes principais através da Análise de Componentes Principais (PCA). A primeira componente principal (PC1), a qual explicou 66,1% da variância total, foi adotada como índice de tamanho corporal e utilizada para ordenar os indivíduos do maior para o menor dentro do universo de cada sexo. A partir disso, foram estimados pontos de corte de quartis de 20% para cada sexo, de modo que os indivíduos presentes no primeiro nível (0−20%) foram tratados como pequenos, aqueles entre 40 e 60% foram tratados como intermediários, e os presentes no último nível (80−100%) foram tratados como grandes (Material Suplementar 1 − Anexo 3).

Caracterização dos ninhos de atobás-marrons no ASPSP

Todos os ninhos ativos e não-ativos, ou seja, contendo material de ninho como pequenas pedras e penas, foram georeferenciados através do método de posicionamento cinemático em tempo real (RTK) com uma base fixa e uma estação móvel (Topcon Positioning Systems Inc.), a qual foi colocada no ponto central de cada ninho. A

comunicação por rádio entre a base e a estação móvel fornece correções em tempo real das posições quando trianguladas com satélites de GPS, viabilizando a obtenção de dados de altitude, latitude, e longitude com resolução aproximada de 20 mm. Após a obtenção de 304 pontos em 1580 m^2, foi possível construir um mapa tridimensional da colônia com uma resolução de 0,19 pontos/m^2. Além disso, os ninhos foram fotografados e numerados digitalmente para a identificação em campo.

Por conta da heterogeneidade da paisagem da colônia, cada ninho foi classificado quanto à sua qualidade seguindo uma combinação de parâmetros, como altitude global, distância e altitude em relação aos ninhos vizinhos, distância da borda da colônia, e suscetibilidade de ser destruído por ondas (Figura 2 − Anexo 3). Um ponto de corte de 15 m acima do nível do mar foi estabelecido como critério de qualidade, de modo que os ninhos acima dos 15 m de altitude foram classificados como ninhos de alta qualidade pelo fato de estarem protegidos das ondas, mesmo durante tempestades. Durante um evento extremo em 2014, todos os ninhos abaixo dos 15 m de altitude foram destruídos por ondas, representando quase 80% do total de ninhos ativos (observação pessoal). A reprodução colonial é considerada uma estratégia contra predadores, pois tem sido demonstrada uma maior sobrevivência de filhotes em ninhos mais distantes da borda da colônia (Forster & Phillips, 2009; Minias, 2014). Neste estudo, foram considerados ninhos periféricos, arbitrariamente, aqueles localizados a menos de 3 m da borda da colônia. Além disso, entre os ninhos periféricos, há ninhos que são frequentemente destruídos por ondas durante marés de sizígias na face sul da colônia, os quais foram classificados como ninhos de baixa qualidade. A posição em relação aos ninhos vizinhos foi definida pela comparação da altitude de cada ninho em relação aos três ninhos mais próximos, o que fornece informação sobre a possibilidade do ninho ser inundado pelo

acúmulo de água da chuva, deixando ovos e filhotes encharcados. Portanto, ninhos periféricos com a menor altitude em relação aos três vizinhos mais próximos foram classificados como ninhos de baixa qualidade. Para cada ninho, as distâncias em relação aos três ninhos mais próximos foram medidas e transformadas na distância média entre ninhos. A média da distância média entre ninhos para ninhos com menos de 15 m de altitude foi 1,2 m e, portanto, ninhos não periféricos e não sendo o mais baixo em comparação com os vizinhos, mas com distância média entre ninhos acima de 1,2 m foram classificados como ninhos de alta qualidade.

Rastreamento remoto

Para estudar as viagens de alimentação de atobás-marrons, foram utilizados GPS miniaturizados (12,5 g, 19 × 25 × 5 mm) com antena integrada e bateria recarregável (Technosmart Europe), configurados para obter uma posição por segundo quando o rastreamento foi de apenas um dia, e uma posição a cada 10 segundos quando o aparelho foi fixados para dois dias de rastreamento. Durante 30 dias em julho de 2015, os atobás foram capturados nos ninhos para colocação dos aparelhos entre 04:00 h e 05:00 h do horário local (UTC-2). Os aparelhos foram impermeabilizados com tubos termocontráteis selados com maçarico e fixados nas três penas centrais da cauda utilizando fita TESA. O peso total de todo o material (*i.e.* tubo, fita, e aparelho) não ultrapassou 3% da massa corporal do atobá mais leve do ASPSP, um macho com 1185 g, como recomendado por Phillips *et al*. (2003) para aves marinhas. Após a recuperação do aparelho, os dados foram transferidos para um computador portátil através do programa GiPSy-4 (Technosmart Europe). A partir dos dados brutos, foram extraídos alguns parâmetros das viagens, como

distância total viajada (D), máxima distância da colônia (Dmax), duração da viagem (T) e sinuosidade (D/2Dmax). A colocação e recuperação (quando as amostras biológicas foram coletadas) de cada aparelho demorou ± 5 e 10 min, respectivamente, e, após o manuseio, a ave foi imediatamente liberada no ninho onde foi capturada.

Análises estatísticas

Anexo 1

Diferenças fenotípicas entre as colônias foram testadas de forma multivariada com MANOVA, e T^2 de Hotelling como *post-hoc*. Os resíduos foram utilizados para testar normalidade univariada, com o teste de Shapiro-Wilk, e multivariada, com os testes de Mardia, Henze-Zirkler, e Royston, os quais estão implementados no pacote *MVN* do programa R (Korkmaz *et al*., 2014; R Core Team, 2016). A dissimilaridade entre as populações foi quantificada com distância Euclidiana baseada no conjunto multivariado de dados (cúlmen, tarso, asa, e massa corporal) com valores padronizados. Baseado nas distâncias Euclidianas obtidas, dendrogramas foram gerados utilizando o pacote *ggdendro* do programa R. Um índice de tamanho corporal foi obtido através da adoção da primeira componente principal (PC1) de uma PCA com valores padronizados de cúlmen, tarso e asa. Além disso, modelos lineares generalizados (GLM) com distribuição normal foram utilizados para demonstrar quais variáveis ambientais melhor explicavam a variância dos dados de tamanho e massa corporal.

Anexo 2

Índices de diversidade genética, como heterozigozidades observada e esperada, desvios do equilíbrio de Hardy-Weinberg (Nei, 1978), e riqueza alélica, foram calculados para as seis colônias de atobás-marrons no programa Arlequin 3.5 (Excoffier & Lischer, 2010). A distância genética entre as populações foi calculada através do F_{ST} de Nei (1977) com o programa GenAlEx 6.5 (Peakall & Smouse, 2012), e diferenças genotípicas pareadas foram testadas com o teste exato de Fisher, no programa Genepop 4.4 (Rousset, 2008). Uma árvore filogenética foi construída utilizando o método UPGMA (Sneath & Sokal, 1973) no programa POPTREE2 (Takezaki *et al*., 2010).

A estruturação populacional entre as seis colônias estudadas foi testada através de duas técnicas, uma multivariada e uma Bayesiana. A técnica multivariada foi a Análise de Coordenadas Principais (PCoA), a qual foi realizada no programa GenAlEx 6.5 com dados padronizados, a fim de identificar, visualmente, similaridades e agrupamentos entre colônias. Além disso, foi conduzida a análise de agrupamento Bayesiano implementada no programa STRUCTURE 2.3.4, a qual determina o número mais plausível de grupos (K) (Pritchard *et al*., 2000). Números de K de 1 a 10 foram testados através de 20 corridas independentes para cada K com 100.000 passos de *burn-in* e 1.000.000 cadeias de Markov. O cálculo de ΔK (Evanno *et al*., 2005) foi utilizado para detectar o melhor número de K no programa STRUCTURE HARVESTER Web 0.6.94 (Earl & vonHoldt, 2012). As 20 corridas independentes para o melhor K foram mescladas com o programa CLUMPP 1.1.2 (Jakobsson & Rosenberg, 2007) e um gráfico de barras foi gerado com o programa DISTRUCT 1.1 (Rosenberg, 2004). Por fim, uma inferência Bayesiana de fluxo

gênico bidirecional foi conduzida utilizando o programa BAYESASS 1.3 (Wilson & Rannala, 2003).

O modelo de Isolamento por Distância foi testado através da correspondência de matrizes de distâncias geográfica e genéticas pareadas, com o teste de Mantel. O modelo de Isolamento pelo Ambiente também foi testado através do teste de Mantel com a matriz de distância genética e as matrizes com distâncias de Mahalanobis para cada variável ambiental considerada (temperatura do ar, temperatura superficial do mar, concentração de clorofila α, distância da costa, densidade populacional, e amplitude de nicho isotópico). Além disso, duas abordagens complementares foram aplicadas para identificar a melhor combinação de variáveis ambientais para explicar a estrutura genética de atobás-marrons. A primeira é um GLM Bayesiano implementado no programa GESTE 2.0, de modo que as características ambientais foram inseridas como variáveis independentes para explicar as distâncias F_{ST} população-específicas (Foll & Gaggiotti, 2006). A segunda abordagem foi uma Análise de Redundância, uma abordagem multivariada baseada no pressuposto de que a relação entre as frequências alélicas e os dados ambientais é linear. O pacote *vegan* do programa R foi utilizado para identificar a contribuição de cada variável ambiental sobre a variação genética através da função *ordistep* em um procedimento passo a passo iniciando com o modelo saturado (*backward*), selecionando como melhor modelo aquele com o menor valor do Critério de Informação de Akaike (AIC). *P*-valores foram calculados baseados em 10.000 permutações.

Anexo 3

Razões isotópicas de carbono e nitrogênio obtidas do soro sanguíneo dos atobás-marrons foram utilizadas para calcular a amplitude do nicho isotópico através da rotina Bayesiana implementada no pacote SIBER (Jackson *et al*., 2011). O pacote foi utilizado para calcular a área padrão das elipses ($‰^2$) corrigida para pequenos tamanhos amostrais (SEAc) para cada categoria de tamanho corporal, e também para calcular a porcentagem de sobreposição entre as elipses. As proporções de contribuição de cada presa à dieta de cada grupo foram estimadas como modelos mistos Bayesianos através da rotina implementada no pacote MixSIAR (Stock & Semmens, 2013) no programa R. Para isso, as razões isotópicas das três presas mais importantes encontradas nas análises de material regurgitado (conforme abaixo) foram utilizadas como fonte. Os fatores de enriquecimento trófico utilizados para carbono e nitrogênio foram aqueles propostos para aves marinhas piscívoras com tecido sem lipídio: 1.1 ± 0.5 para $\delta^{13}C$, e 2.8 ± 0.5 para $\delta^{15}N$ (Bearhop *et al*., 2002). Distribuições posteriores para cada presa para cada grupo foram obtidas após 1.000.000 cadeias de Markov, descartando as primeiras 500.000 como *burn-in* (Stock & Semmens, 2013).

A caracterização da dieta dos atobás-marrons a partir do material regurgitado foi realizada com o Índice de Importância Relativa Presa-Específico (PSIRI), o qual é baseado em parâmetros presa-específicos (ou seja, variando de > 0% até 100%), como frequência de ocorrência, abundância, e massa (Brown *et al*., 2012). Devido ao fato de que nem todos os itens estavam no mesmo estágio de digestão no momento da coleta, a massa individual foi estimada através de uma regressão Bayesiana previamente publicada

para peixes de corpo alongado, considerando o comprimento da cabeça até a forquilha da cauda (Froese *et al*., 2014).

Diferenças de tamanho corporal entre categorias de qualidade de ninho foram testadas com ANOVA, sendo que a normalidade e homoscedasticidade dos resíduos foram testadas com testes de Shapiro-Wilk e Levene, respectivamente. Diferenças intersexuais dos parâmetros das viagens de forrageio e das razões isotópicas foram testadas com o teste não-paramétrico de Mann-Whitney. Diferenças intrassexuais de comportamento de forrageio e dieta foram testadas com o teste de Kruskal-Wallis, utilizando os grupos de tamanho corporal como fatores. Além disso, foram realizados testes de correlação entre o índice de tamanho corporal contínuo (ou seja, sem separar por grupos) e os parâmetros da dieta e das viagens de forrageio com o tau de Kendall (τ), o qual mede correlação entre duas variáveis ordenadas (Legendre & Legendre, 2012). Finalmente, árvores de regressão, baseadas na seleção aleatória dos dados e das variáveis, foram utilizadas para identificar a importância do tamanho corporal dos atobás-marrons para explicar a variância de cada parâmetro do comportamento de forrageio e da dieta. A classificação das variáveis de acordo com sua importância para cada árvore de regressão foi realizada com o algoritmo Random Forest, implementado no pacote *randomForest* (Liaw & Wiener, 2002) no programa R, gerando 1000 árvores de regressão com 20 permutações por árvore para dados *out-of-bag*. A classificação é realizada através do ordenamento das variáveis de acordo com a taxa de erro que a remoção de cada variável causa para o modelo como um todo, ou seja, quanto maior o erro causado pela remoção de uma determinada variável para o modelo, maior a importância dessa variável.

RESULTADOS

Diferenciação fenotípica (Anexo 1)

No total, 135 machos e 141 fêmeas de atobás-marrons foram amostrados para este estudo. Como esperado, foi observado dimorfismo sexual reverso para todas as variáveis amostradas e em todas as colônias, sendo que fêmeas foram, em média, 5,2% maiores e 21,9% mais pesadas do que machos (Tabela 2 − Anexo 1). Tanto para machos como para fêmeas, os indivíduos amostrados em Fernando de Noronha e no Atol das Rocas foram os menores e mais leves e, ao contrário do esperado, os machos amostrados no ASPSP foram os maiores e mais pesados, e as fêmeas foram as mais pesadas (Figura 2 − Anexo 2).

Houve diferença fenotípica significativa entre todas as populações para ambos os sexos (MANOVA − fêmeas: λ de Wilk = 0,061; GL = 5; $p < 0,001$; machos: λ de Wilk = 0,041; GL = 5; $p < 0,001$). No entanto, o teste utilizado como *post-hoc* não demonstrou diferenças significativas entre Cagarras e Moleques do Sul para ambos os sexos, e entre Moleques do Sul e ASPSP para fêmeas (Tabela 3 − Anexo 1). As maiores distâncias Euclidianas multivariadas foram observadas entre Fernando de Noronha/Atol das Rocas e ASPSP (4,472 em média), e a menor distância Euclidiana multivariada foi observada entre Cagarras e Moleques do Sul (0,315). As distâncias Euclidianas médias entre as colônias costeiras, e entre as colônias costeiras e o ASPSP, foram relativamente baixas: 0,912 e 1,493, respectivamente (Tabela 3 − Anexo 1). O agrupamento utilizando distâncias Euclidianas baseadas em dados fenotípicos multivariados gerou dendrogramas com dois grupos: um ramo com os atobás-marrons menores e mais leves (*i.e.* Fernando

de Noronha e Atol das Rocas) e outro ramo com os atobás das colônias restantes (Figura 3 − Anexo 1).

Quando a temperatura do ar foi utilizada como variável explicatória nos modelos de regressão para todos os conjuntos de dados, a porcentagem da deviância explicada de tamanho e massa corporais variou de 0 a 2,3%, enquanto que no cenário sem o ASPSP a deviância explicada do modelo com temperatura do ar como variável explicatória variou de 50,6 a 72,9% (Figura 4 − Anexo 1). Em geral, em ambos os cenários para todos os conjuntos de dados, os modelos foram mais explicativos (*i.e.* menor AIC e maior porcentagem da deviância explicada) quando clorofila α e temperatura superficial do mar foram adicionadas (Tabela A1 − Anexo 1). Por fim, o modelo com temperatura do ar e a interação entre clorofila α e temperatura superficial do mar (= temperatura do ar + (clorofila α * temperatura superficial do mar)) teve a maior porcentagem de deviância explicada (80,4%) e o menor AIC para todos os conjuntos de dados em ambos os cenários, *i.e.* com e sem o ASPSP.

Estrutura populacional (Anexo 2)

Um total de 119 atobás-marrons foram amostrados para este estudo, variando de 18 indivíduos em Moleques do Sul a 24 indivíduos no ASPSP. Não foram detectados erros de genotipagem pelo programa MICRO-CHECKER, e apenas 2 de 54 *loci* (6 populações × 9 *loci*) desviaram-se do equilíbrio de Hardy-Weinberg. Não foi detectado desequilíbrio de ligação para qualquer par de loci em qualquer população. Os nove *loci* foram polimórficos e apresentaram riqueza alélica média de 2,6 alelos, mas os atobás-marrons do ASPSP apresentaram baixo polimorfismo (44,4%) quando comparados aos

atobás do Atol das Rocas, Abrolhos, Cagarras (88,9%) e aos atobás de Fernando de Noronha e Moleques do Sul (100%). Os atobás-marrons do ASPSP também apresentaram a menor heterosigozidade observada (Tabela 1 − Anexo 2) e uma baixa porcentagem de alelos raros (~8%). O coeficiente de endocruzamento (F_{IS}) foi próximo de zero para todas as populações.

O gráfico da PCoA resultou nas coordenadas 1 e 2 que explicaram 73,9% e 18,0% da variância genética total, respectivamente. A coordenada 1 separou o ASPSP das demais colônias, enquanto que a coordenada 2 manteve agrupados Fernando de Noronha e Atol das Rocas, mas separou estas das colônias costeiras (Figura S2 − Anexo 2). Portanto, os gráficos de barra gerados a partir dos resultados do programa STRUCTURE foram construídos considerando tanto K = 2 quanto K = 3 (Figura 1b − Anexo 2; veja também a Figura S3 − Anexo 2 para os resultados gerados pelo programa STRUCTURE HARVESTER). A árvore filogenética construída pelo método UPGMA foi consistente com a PCoA, visto que sugeriu o agrupamento das seis colônias em dois ramos, mas com uma divisão mais fraca entre o grupo Fernando de Noronha/Atol das Rocas e as colônias costeiras (Figura 1c − Anexo 1). Os menores valores de F_{ST} foram observados entre Cagarras e Moleques do Sul, e entre Atol das Rocas e Fernando de Noronha, para os quais também não foram observadas diferenças genotípicas significativas (Tabela 2 − Anexo 2). Portanto, foi possível distinguir de maneira forte dois grupos entre as seis colônias (ASPSP *vs.* as demais colônias), mas com uma diferenciação sutil entre as colônias costeiras e Fernando de Noronha/Atol das Rocas. As maiores taxas de fluxo gênico foram observadas dentro dos grupos, mas uma proporção relevante de migrantes também ocorreu entre Moleques do Sul e Fernando de Noronha/Atol das Rocas (Figura 3 − Anexo 1).

As variáveis ambientais foram altamente heterogêneas (Figura 2 − Anexo 2; Figura S4 − Anexo 2). Foi observada correlação significativa entre as variáveis ambientais clorofila α *vs*. temperatura superficial do mar, clorofila α *vs*. temperatura do ar, e temperatura superficial do mar *vs*. temperatura do ar ($p < 0,01$; Tabela S1 − Anexo 2) e, portanto, apenas clorofila α foi utilizada nas análises posteriores para evitar multicolinearidade. Correspondência entre as matrizes de distância genética e geográfica foi positiva, mas não significativa ($R^2 = 0,202$; $p = 0,187$), enquanto que a correspondência entre as matrizes de distâncias genética e de distância da costa (referida no Anexo 2 como '*DisCoast*') foi positiva e significativa ($R^2 = 0,925$; $p < 0,01$; Tabela S2 − Anexo 2). A probabilidade posterior foi baixa em todos os GLM Bayesianos. O modelo nulo teve a menor probabilidade, enquanto que o GLM ajustado com 'densidade populacional + distância da costa' teve a maior probabilidade posterior (Tabela S3 − Anexo 2). O modelo saturado (distância da costa + clorofila α + densidade populacional + amplitude de nicho isotópico) foi significativo para explicar a variabilidade genética na análise de redundância ($p = 0,027$), mas o modelo de maior parcimônia conteve apenas 'densidade populacional + distância da costa' ($p = 0,008$) (Figura 4 − Anexo 2).

Diferenças intrapopulacionais nos atobás-marrons do ASPSP (Anexo 3)

No total, 319 atobá-marrons (160 fêmeas e 159 machos) foram medidos quanto ao tamanho e massa corporais. Em média, fêmeas foram maiores e mais pesadas do que machos: 5,1% para comprimento do cúlmen, 7,1% para comprimento do tarso, 4,4% para corda da asa, e 25,3% para massa corporal (Tabela 1 − Anexo 3). De 2014 a 2015, a fidelidade ao ninho foi de ~70% ($n = 90$), e a fidelidade de parceiro foi de ~90% ($n = 37$).

Os valores de isótopos estáveis variaram de -17,52 a -16,82‰ para $\delta^{13}C$, e de 11,24 a 13,33‰ para $\delta^{15}N$, sendo que fêmeas apresentaram valores significativamente maiores tanto para carbono como para nitrogênio ($p < 0,05$). Amplitude de nicho isotópico foi similar para machos e fêmeas, e as elipses Bayesianas de cada sexo foram sobrepostas em 21%.

No total, foram analisados 72 conteúdos estomacais e 307 itens alimentares de 60 indivíduos distintos, sendo que a assíntota de riqueza de espécies das presas foi atingida após a análise de 21 conteúdos estomacais e 55 itens. Todas as sete presas foram identificadas em nível de espécie, e o peixe-voador *Exocoetus volitans* foi a espécie mais importante (PSIRI = 62,8%), seguido por outro peixe-voador, *Oxyporhamphus micropterus* (PSIRI = 17,6%) (Tabela 1 − Anexo 3). Devido à sua alta importância presa-específica, os espécimes de *E. volitans* foram divididos em três categorias, para as análises de contribuição à dieta com dados isotópicos (MixSIAR) e material regurgitado (PSIRI), de acordo com seu comprimento da cabeça à forquilha da cauda: indivíduos < 100 mm foram considerados pequenos, indivíduos entre 100 e 150 mm foram considerados intermediários, e indivíduos > 150 mm foram considerados grandes. Não foram observadas diferenças substanciais entre os sexos para o PSIRI, mas *E. volitans* grande teve maior importância para fêmeas, enquanto que *E. volitans* intermediário e *O. micropterus* foram mais importantes para machos (Material Suplementar 3 − Anexo 3). Os modelos de mistura Bayesianos conduzidos no MixSIAR considerando como fontes os três itens alimentares mais importantes (*i.e. E. volitans* grandes e intermediários, e *O. micropterus*), apresentaram resultados similares, sendo que as probabilidades posteriores de assimilação de presas pelas fêmeas foram de 69,7% para *E. volitans* grandes, 7,2%

para *E. volitans* intermediários, e 23,1% para *O. micropterus*, enquanto que para machos foram 59,1%, 5,9%, e 35%, respectivamente (Figura 3 − Anexo 3).

O comportamento de forrageio foi registrado para 97 indivíduos (52 fêmeas e 45 machos), totalizando 258 viagens de forrageio. A duração média das viagens foi de 57 ± 27,5 min, enquanto que as médias para distância máxima da colônia e distância total percorrida foram 7,1 ± 4,3 km e 27 ± 13,7 km, respectivamente. No entanto, não foram observadas diferenças significativas entre sexos para os parâmetros das viagens de forrageio (Figura 4 − Anexo 3). Com isso, as razões isotópicas de nitrogênio e carbono foram as variáveis mais importantes para discriminar os sexos nas árvores de regressão, seguidas por Dmax, T, D, e Sinuosidade, respectivamente. Não foram observadas diferenças significativas tanto nos parâmetros das viagens de forrageio como nas razões isotópicas de carbono e nitrogênio entre diferentes estágios reprodutivos e, portanto, não foram removidos indivíduos dos conjuntos de dados de machos e fêmeas para as análises subsequentes (Material Suplementar 5 − Anexo 3).

Considerando o mínimo polígono convexo, a área da colônia foi de 601 m^2, o que representa uma densidade de 0,51 ninhos/m^2. A altitude dos ninhos variou de 10,7 a 20,7 m acima do nível do mar, e a distância média entre ninhos foi de 1,03 ± 0,16 m. A altitude dos ninhos foi negativamente correlacionada com a distância média entre os ninhos (τ = -0,15; p = 0,0004), ou seja, a densidade de ninhos foi maior nas áreas mais altas da colônia. Considerando 15 m acima do nível do mar como um ponto de corte, a densidade de ninhos em áreas > 15 m foi de 0,89 ninhos/m^2 (114 ninhos em 128,9 m^2), enquanto que em áreas < 15 m a densidade foi de 0,63 ninhos/m^2 (190 ninhos em 301 m^2). Embora 304 ninhos tenham sido georreferenciados, apenas 112 ninhos estavam ativos durante o estudo e, portanto, apenas esses foram classificados de acordo com sua qualidade. No

total, 26,2% dos 112 ninhos foram classificados como sendo de baixa qualidade, 29,9% de qualidade intermediária, e 43,9% de alta qualidade (Figura 5 − Anexo 3).

Foram detectadas diferenças nas médias de tamanho corporal de fêmeas entre as diferentes categorias de qualidade de ninho (F = 9,67; p = 0,0001), sendo que as diferenças significativas de médias de tamanho corporal estiveram entre ninhos de alta e baixa qualidades (p = 0,0002), e entre ninho de alta e intermediária qualidades (p = 0,02), com fêmeas grandes predominando (76%) em ninhos de alta qualidade. Por outro lado, não foram detectadas diferenças nas médias de tamanho corporal de machos entre as diferentes categorias de qualidade de ninho (Figura 6 − Anexo 3). Em relação ao comportamento de forrageio, não foram observadas diferenças significativas nos valores médios dos parâmetros das viagens de forrageio, obtidos com rastreamento remoto por GPS miniaturizados, entre os grupos de tamanho corporal, tanto para machos como para fêmeas. Também não foram observadas correlações significativas entre o índice de tamanho corporal contínuo e os parâmetros das viagens de forrageio para ambos os sexos (Figura 4 − Anexo 3). Além disso, não foram observadas diferenças nas médias de δ^{13}C e δ^{15}N para ambos os sexos entre os grupos de tamanho corporal, bem como não foi observada correlação significativa entre o índice de tamanho corporal e os valores de isótopos estáveis tanto para machos como para fêmeas. A sobreposição média das elipses Bayesianas dos grupos de tamanho corporal baseadas em valores isotópicos foi de 26% para fêmeas e 24% para machos (Figura 7 − Anexo 3). A riqueza de espécies de presas variou de três a sete espécies entre os grupos de tamanho corporal de machos e fêmeas, sendo que *E. volitans* foi o principal item para todos os grupos de tamanho corporal de ambos os sexos, representando 79,6%, 90,7% e 80,6% da dieta de fêmeas pequenas, médias e grandes, e 57,4%, 41,7% e 76,3% da dieta de machos pequenos, médios, e

grandes, respectivamente (Tabela 1 − Anexo 3). Não houve diferenças substanciais nos valores de PSIRI entre aves com tamanhos corporais pequenos, médios e grandes, tanto para machos como para fêmeas, mas as comparações com dados de PSIRI entre grupos de tamanho corporal para machos teve baixo poder, devido ao pequeno tamanho amostral (Tabela 1 − Anexo 3). Razões isotópicas de carbono e nitrogênio também foram estatisticamente similares entre os grupos de tamanho corporal de machos e fêmeas, assim como as contribuições das fontes nos modelos de mistura Bayesianos (Figura 3 − Anexo 3). Por fim, o tamanho corporal foi a segunda variável mais importante para explicar a variância dos valores de δ^{13}C para fêmeas, de acordo com as árvores de regressão geradas pelo algoritmo Random Forest, e a terceira mais importante para explicar os valores de δ^{15}N para machos, mas para nenhuma das variáveis dependentes o tamanho corporal foi a mais importante variável explicatória (Material Suplementar 6 − Anexo 3).

CONCLUSÕES

As médias de tamanho corporal dos atobás-marrons diferiram significativamente entre as seis colônias estudadas ao longo do gradiente latitudinal no sudoeste do Oceano Atlântico, mas a temperatura do ar não foi suficiente para explicar as diferenças, o que confirma parcialmente a previsão da **Hipótese 1**. Como demonstrado no Anexo 1 e previsto nesta hipótese são necessárias variáveis ambientais complementares para explicar a distribuição da biodiversidade no ambiente marinho como, por exemplo, temperatura superficial do mar e concentração de clorofila α, a qual foi utilizada neste estudo como *proxy* de produtividade primária. No entanto, no cenário em que a colônia do ASPSP foi removida das análises, foi observado um gradiente linear de distribuição

de tamanho corporal em relação à temperatura do ar, demonstrando que essa variável ambiental também possui importância para o ajuste de tamanho corporal, o que, em outras palavras, significa dizer que a Regra de Bergmann pode ser aplicada mesmo em nível intraespecífico em aves marinhas, desde que sejam consideradas variáveis adicionais, como aquelas supracitadas.

A forte diferenciação fenotípica observada entre o ASPSP e Fernando de Noronha/Atol das Rocas sugeriu ausência de fluxo gênico entre essas populações e a existência de características locais promovendo adaptação local, dada a relativamente pequena distância geográfica entre as colônias. Nesse contexto, a similaridade fenotípica entre o ASPSP e as colônias costeiras poderia ser resultado de elevadas taxas de dispersão entre os dois grupos ou, por outro lado, a existência de pressões seletivas similares causando convergência adaptativa.

Quando analisadas em nível molecular, as relações intraespecíficas de atobás-marrons no sudoeste do Oceano Atlântico passam a fazer sentido à luz da heterogeneidade da paisagem marinha e de características intrínsecas de cada colônia, como a densidade populacional. Desse modo, a **Hipótese 2** foi confirmada, visto que o Isolamento pelo Ambiente foi mais plausível para explicar a diversidade genética em relação ao modelo de Isolamento pela Distância. Os atobás-marrons do ASPSP apresentaram um alto número de homozigotos, sugerindo isolamento populacional em relação às demais colônias, o que foi comprovado pelas estimativas de taxa de dispersão e de estruturação populacional multivariada e Bayesiana. As peculiaridades da colônia do ASPSP parecem ser as principais causas do isolamento e diferenciação populacional, reforçando a ideia de que os atobás-marrons, embora possuam alta mobilidade, apresentam uma tendência à adaptação local, representando um mecanismo crucial para manutenção da diversidade

intraespecífica nesta espécie, como também tem sido sugerido para outras espécies de aves marinhas.

Embora mais fraco, o agrupamento das colônias costeiras em um único *cluster* vai ao encontro do exposto acima, visto que as similaridades ambientais, especialmente a localização sobre a plataforma continental e dependência dos recursos ali existentes, poderiam ser a causa das elevadas taxas de dispersão observadas entre estas colônias. O mesmo ocorre com as colônias de Fernando de Noronha e Atol das Rocas, as quais estão separadas por apenas 150 km e são influenciadas pelas mesmas águas oligotróficas da Corrente Sul Equatorial. Teias tróficas marinhas tendem a apresentar uma correlação negativa entre a disponibilidade de nutrientes e o número de níveis tróficos, ou seja, em ambientes com baixa disponibilidade de nutrientes, os organismos tendem a apresentar menor tamanho corporal, o que aumenta o número de níveis intermediários entre a base e o topo, ocasionando maior dissipação de energia ao longo do processo e, consequentemente, viabilizando predadores de topo com menor tamanho corporal. Portanto, nos Anexos 1 e 2 é fornecida uma discussão detalhada sobre as diferenças na paisagem marinha entre as colônias costeiras e Fernando de Noronha/Atol das Rocas, as quais foram sugeridas como principais fatores de diferenciação fenotípica e genotípica entre esses dois *clusters*.

A ampla amostragem da população de atobás-marrons no ASPSP realizada para o Anexo 3 demonstrou que as características da paisagem da colônia promovem seleção de fenótipos. De fato, a peculiar densidade de ninhos e a heterogeneidade da paisagem da colônia sugerem haver alguma organização hierárquica na escolha do local para nidificação, ao mesmo tempo em que a abundante oferta de alimento no entorno imediato do arquipélago sugere que a pressão seletiva esteja mais em terra do que no mar. Por isso,

a **Hipótese 3** foi confirmada parcialmente, visto que não foram observadas diferenças na dieta e no comportamento de forrageio entre os diferentes grupos de tamanho corporal, tanto para machos, como para fêmeas. Por sua vez, a diferença de tamanho corporal de fêmeas entre as categorias de qualidade de ninho indicaram a existência de uma pressão seletiva relacionada à conquista e defesa de territórios. A nidificação em territórios altos, ou em áreas protegidas das ondas, da chuva, e dos próprios vizinhos conspecíficos, pode ser crucial para o sucesso da reprodução no ASPSP.

Portanto, as colônias de atobás-marrons localizadas no sudoeste do Oceano Atlântico demonstraram ser uma promissora área para o estudo da ecologia evolutiva e, especialmente, dos processos microevolutivos que moldam populações naturais. A diversidade biológica dos lagartos *Anolis* nas Ilhas do Caribe, dos tentilhões de Darwin nas Ilhas Galápagos, e dos peixes *guppy* nos rios da América Central, tem sido explorada sob o ponto de vista evolutivo nas últimas décadas e, atualmente, o monitoramento de médio e longo prazo desses organismos representa a base do conhecimento da influência dos fatores ambientais sobre os processos microevolutivos em populações naturais. O conhecimento atual sobre os processos microevolutivos agindo sobre os predadores de topo marinhos são incipientes e pouco conclusivos. No entanto, é interessante notar que mesmo espécies estritamente marinhas tenham fenótipos selecionados por características ambientais terrestres, como também vem sendo observado para outras espécies de aves e mamíferos marinhos. Além disso, a alta filopatria observada nesses grupos facilita a identificação da associação de características da paisagem das áreas reprodutivas com a diversidade biológica, o que, auxiliado pelas ferramentas de estudo atualmente disponíveis, torna viável o estudo da ecologia de paisagem e da dinâmica eco-evolutiva com a megafauna marinha.

PERSPECTIVAS FUTURAS

O presente estudo forneceu importantes informações práticas e teóricas de interesse local e global. A identificação das unidades evolutivas de atobás-marrons representa uma informação valiosa para a definição de futuras ações práticas para a conservação da espécie no Brasil. Além disso, a ilustração do modelo de Isolamento pelo Ambiente em aves marinhas, bem como a combinação de ferramentas e técnicas analíticas utilizadas, representa um exemplo a ser aplicado para qualquer espécie em distintos conjuntos de condições ambientais. Nesse contexto, algumas perspectivas de aplicação do conhecimento gerado no presente estudo são elencadas a seguir:

- Comparar os fenótipos e genótipos de atobás-marrons amostrados neste estudo com outras colônias do Oceano Atlântico, como a Ilha Ascensão, o Arquipélago de Cabo Verde, e as ilhas do Caribe. Uma análise de genômica da paisagem esclareceria a conectividade em diferentes escalas de tempo, fornecendo informações que vão desde a direção do fluxo gênico no período de ocupação do Oceano Atlântico, passando por eventos de redução populacional, associados às mudanças climáticas do passado e, por fim, prevendo alterações na estruturação populacional com as mudanças climáticas previstas para as próximas décadas;
- Monitorar a médio e longo prazo as colônias estudadas nesta tese, realizando amostragens em uma mesma janela temporal, e considerando variações sazonais, de material para análises de isótopos estáveis e de dados de distribuição espacial durante viagens de forrageio. Com isso, será possível identificar oscilações dentro de cada colônia nas interações presa-predador e refinar o conhecimento sobre o papel do ambiente na diferenciação populacional de atobás-marrons. Para uma análise mais refinada, poderiam

ser incluídos elementos adicionais nas análises de isótopos estáveis, como enxofre, oxigênio e hidrogênio, e também equipamentos de rastreamento que fornecessem informações comportamentais mais finas, como acelerômetros tri-axiais com registrador de profundidade de mergulho, os quais poderiam estar associados a estimadores indiretos de orçamento energético, como água duplamente marcada ou gravador de frequência cardíaca;

- Rastrear os atobás-marrons das colônias estudadas durante o período não reprodutivo. A distribuição em período não reprodutivo tem sido atribuída como um importante mecanismo de diferenciação populacional (Friesen *et al.*, 2007; Friesen, 2015), ou seja, o fluxo gênico entre duas colônias é mais provável quando os indivíduos dessas colônias se encontram, o que pode ocorrer nas áreas de invernagem. Atualmente, há equipamentos miniaturizados desenvolvidos para isso, como geolocalizadores ou GPS miniaturizados equipados com placas solar, o que estende a duração da bateria;
- Monitorar em médio e longo prazo os atobás-marrons do ASPSP em relação à ocupação dos territórios na colônia, fidelidade de ninho, fidelidade ao parceiro, e frequência de reprodução, utilizando como base o mapeamento de ninhos realizado neste estudo. Além disso, é necessário considerar outras variáveis individuais que podem influenciar na conquista de territórios de alta qualidade como, por exemplo, a personalidade e a idade dos indivíduos;
- Identificar o papel da comunidade de aves marinhas de cada arquipélago sobre as características comportamentais e de dieta dos atobás-marrons. Isso implica em obtenção de informação massiva em médio e longo prazo sobre o uso do espaço e dos recursos por outras espécies de aves marinhas ocorrendo nos arquipélagos estudados;

- Por fim, testar a aplicabilidade do modelo de Isolamento pelo Ambiente em outras espécies de aves marinhas que possuam colônias de fácil acesso em distintos arquipélagos na costa brasileira, como é o caso de *Sula dactylatra, Fregata magnificens, Anous stolidus, Anous minutus* e *Onychoprion fuscatus*.

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ANEXOS

ANEXO 1

When Bergmann's rule fails: evidences of environmental selection pressures shaping phenotypic diversification in a widespread seabird

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Organisms tend to exhibit phenotypes that can be shaped by climate, commonly demonstrating clinal variations along latitudinal gradients. In vertebrates, air temperature plays a major role in shaping body size in both ectothermic and endothermic animals. However, additional small-scale environmental factors can also act as selection pressures in the marine ecosystem (e.g., primary productivity), evidencing multi-scale processes acting on marine organisms. In this study, we tested Bergmann's rule in a widely distributed seabird, the brown booby (Sula leucogaster), in addition to evaluating the relationship of sea surface temperature and chlorophyll α with phenotypes. We used traits from a morphometric dataset (culmen, wing chord, and tarsus length) and body mass of 276 brown boobies distributed on six breeding sites along a latitudinal gradient in the South Atlantic Ocean (0–27°S). We found significant differentiation among colonies, but phenotypic similarities were observed between colonies located at the extremes of the latitudinal gradient. As the colony nearest to the Equator, Saint Peter and Saint Paul archipelago, had the largest and heaviest individuals, the model containing only air temperature explained < 5% of the allometric variation, providing no substantial support for Bergmann's rule. However, when we added the interaction of chlorophyll α and sea surface temperature the deviance explained rose to over 80%. Primary productivity and sea surface temperature do not follow a latitudinal gradient in the ocean and, therefore, the role of small-scale oceanographic processes in shaping body size and the importance of considering additional environmental variables when testing Bergmann's rule in marine organisms are evident.

Birds tend to exhibit phenotypic diversity on an intraspecific level along a climatic gradient, with larger body sizes occurring in colder environments, a phenomenon referred to as Bergmann's rule (James 1970). In general, population structuring among conspecifics results from the selection of multiple pressures to which an organism is exposed (Mayr 1956), such as differences of local oceanographic features around distinct seabird colonies (Friesen 2015). Identifying these variations is relevant both to understanding evolutionary mechanisms of divergence, which may be the first step towards new races (Rensch 1938), and to developing successful conservation strategies, especially in the age of global climate change (Quillfeldt and Masello 2013, Brommer et al. 2015).

Intraspecific population differentiation in seabirds has been frequently observed, either in genotypes (Hailer et al. 2010, Morris-Pocock et al. 2011), foraging behavior (Grémillet et al. 2004, Wakefield et al. 2013), isotopic niche (Wiley et al. 2012, Mancini et al. 2014), or morphometrics (Le Corre and Jouventin 1999, Bertellotti et al. 2002). The majority of over 200 studies using molecular markers have demonstrated gene flow limitation among seabird populations, assuming physical (e.g., ice or land) and non-physical barriers (e.g., philopatry and/or non-breeding distribution) as causes of isolation (Friesen 2015). Although historical or contemporary land barriers are commonly assumed to be one cause of allopatric speciation among seabird sister species (Friesen et al. 2007), several studies have demonstrated intraspecific differentiation even among geographically close colonies with no land barriers (e.g., Hailer et al. 2010, Wiley et al. 2012; reviewed by Friesen 2015).

In a global perspective, the long-standing hypothesis on natural selection pressures for optimum body size suggests increasing size towards high latitudes in direct association with heat conservation/dissipation (Mayr 1956). Although the concordance

of body size with air temperature may have no direct causal relationship, temperature has an indirect effect on the body primarily by influencing other environmental factors (e.g., food supply) (James 1970). This large-scale clinal pattern of variation has been tested in several terrestrial organisms, such as mammals (Clauss et al. 2013, Martinez et al. 2013), birds (James 1970, Ashton 2002), and even in ectothermic animals, such as lizards (Angilletta-Jr. et al. 2004). However, additional environmental parameters seem to be correlated with phenotypes distribution in the marine realm (e.g., sea surface temperature, primary productivity), as has been suggested for marine fishes (Fisher et al. 2010), molluscs (Berke et al. 2013), crustaceans (Timofeev 2001) and even for seabirds (Yamamoto et al. 2016). These small-scale variables can be influenced by local oceanographic processes and not follow a latitudinal gradient (Longhurst et al. 1995), causing deviations of intraspecific and interspecific phenotype differences from the pattern predicted by Bergmann's rule.

The terminology used to describe Bergmann's hypothesis has been a matter of controversy regarding its application in studies at different taxonomic levels. Originally, the existence of a body size-temperature relationship was raised by Bergmann (1847) by referring to the differences observed between "similar organizations" (as translated by Clauss et al. 2013). However, Blackburn et al. (1999) suggested the use of "James's rule" in studies performed at an intraspecific level, referring to James (1970), in which the author demonstrated intraspecific clinal variations in the wing measurements of 12 bird species. Here, we use the term "Bergmann's rule" specifying the taxonomic level we are dealing with, as it represents an illustrative example of the pattern first identified by Carl Bergmann. In addition, confusing interpretations related to terminology can be avoided by following the 'traditional' use from similar studies.

Despite this large-scale well-established rule, seabirds have great flight capability and can travel thousands of kilometers during their seasonal movements, crossing environments with very different conditions (e.g., Shaffer et al. 2006, Egevang et al. 2010). For example, Arctic terns Sterna paradisaea travel ~90,000 km during the non-breeding period, holding the longest animal migration ever reported (Fijn et al. 2013). Furthermore, foraging trips of breeding Cory's shearwater Calonectris diomedea can exceed 7,000 km in just a few days (Ceia et al. 2014). This peculiar high mobility and gene flow limitation, even among geographically close colonies (Friesen 2015), represent a paradox that sheds light on other mechanisms of population differentiation.

Dispersal to other colonies is widely known to be reduced in seabirds due to the high natal philopatry observed in this group (Schreiber and Burger 2001). Resident birds have been observed to present even stronger natal philopatry compared to migratory species, illustrating the influence of ecological factors (i.e. local adaptation) on the dispersal costs (Weatherhead and Forbes 1994). Therefore, remaining in the colony surroundings throughout the year could avoid contact with distinct environmental conditions, positively selecting individuals best fitted to the local conditions and generating diversity from phenotype and genotype shaping (Greenwood 1980, Friesen 2015). Ocean turbulence at very small spatial scale has long been shown to aggregate seabirds (Haney 1987), by modifying the seascape structure and providing food concentration that support top predators through bottom-up processes (Bertrand et al. 2014). Therefore, small-scale ecological hotspots around colonies of resident seabirds would be expected to intensify specialization for local resources, which in turn could lead to the divergence even between nearby populations facing distinct environmental pressures.

Currently, it is recognized that local adaptation is the main barrier to gene flow in animals (Sexton et al. 2014), owing to the fact that organisms facing distinct environmental conditions are subject to different selection pressures (Wright 1943). In the marine realm, local primary productivity tends to influence energy transfer patterns, with productive environments having fewer trophic levels and larger top predators, because there is less energy loss along the trophic web (Pinet 2009). However, marine primary productivity has no a direct relationship with latitude (Longhurst et al. 1995), being influenced primarily by multi-scale physical processes which shape seascape structure (Bertrand et al. 2014). In this context, it would be expected that body size and mass are driven both by large-scale (e.g., air temperature) and small-scale (e.g., primary productivity) environmental variables, with larger and heavier individuals occurring in productive and high latitude areas.

Furthermore, it has been a long-standing theory that ecology drives evolution (e.g., Darwin 1859); however, the importance of eco-evolutionary feedback, a reciprocal causal pathway between ecology and evolution, has only recently been demonstrated (e.g., Agrawal et al. 2013, Duckworth and Aguillon 2015). For instance, global ocean warming would be expected initially to affect baseline trophic levels of the marine ecosystem (Grémillet and Boulinier 2009), favoring seabirds with warm-water preferences and increasing predation on their preferential prey (Quillfeldt and Masello 2013). This should lead to top-down and bottom-up controlling processes occurring simultaneously (Chambers et al. 2011). Therefore, knowing both the extant biodiversity (Taylor and Friesen 2012) and the mechanisms of diversification, which are still poorly understood in seabirds (Friesen 2015), is critical to understand how seabirds and other organisms interact with the seascape structure, and also how they will respond to environmental changes.

In this context, seabirds arise as great organisms to test population differentiation, because they are distributed in breeding aggregations (colonies, mostly on islands) and present high natal philopatry. Furthermore, they depend directly on the marine resources (e.g., fish predation), making it possible to test relationships of multi-scale environmental variables on phenotypes. For this, we used body size and mass from brown booby (Suliformes: Sula leucogaster) colonies distributed along the Southwest Atlantic Ocean, to test clinal phenotypic variations related both to large-scale (latitude and air temperature) and small-scale variables (primary productivity and sea surface temperature). Brown boobies are seabirds distributed in tropical and subtropical regions of all ocean basins, which do not perform true migration (i.e. directed seasonal movements), remaining around their colonies throughout the year (Nelson 2005). In the Southwest Atlantic Ocean they breed in colonies along a latitudinal gradient, but with distinct characteristics regarding seascape structure. Therefore, given the broad latitudinal variation, which implies air temperature variations, we would expect to find strong population differentiation with larger individuals at higher latitudes, consistent with Bergmann's rule. Nevertheless, small-scale oceanographic parameters (i.e. primary productivity and sea surface temperature) can cause deviations in phenotypes from the clinal pattern expected, illustrating the influence of small-scale environmental variables in shaping organisms in the marine ecosystem.

Methods

Study area

We sampled six colonies distributed along a latitudinal gradient ranging from 0–27°S in the Southwestern Atlantic Ocean. Colonies presented distinct distances from the continental coast, ranging from ~4 km to ~1,100 km, and distinct abundances of brown boobies, ranging from ~140 to ~2,500 individuals (Table 1; Fig. 1). Moleques do Sul, Cagarras, and Abrolhos are located over the continental shelf, while Fernando de Noronha, Rocas Atoll, and Saint Peter and Saint Paul archipelago are volcanic in origin, with the latter being part of the Mid-Atlantic ridge. Distances between archipelagos range from ~150 km (Rocas Atoll/Fernando de Noronha) to ~3,900 km (Saint Peter and Saint Paul/Moleques do Sul). All archipelagos are protected by the Brazilian government, and only on Cagarras and on Moleques do Sul there is no permanent human presence. Hereinafter, we refer to Moleques do Sul as "Moleques", to Rocas Atoll as "Rocas", to Fernando de Noronha as "FN", and to Saint Peter and Saint Paul as "SPSP".

The six archipelagos present differences regarding oceanographic dynamics in their surroundings. Coastal sites (Moleques, Cagarras, and Abrolhos) are influenced mainly by shelf waters, such as Tropical Water, which flows southward and is a coastal branch of the Brazil Current (Möller-Jr. et al. 2008). During winter, Moleques is also influenced by the Subtropical Shelf Water, which is generated by the mixing of the Tropical Water with the northward flowing Río de la Plata Plume (Piola et al. 2008). On the other hand, FN, Rocas, and SPSP are influenced by the South Equatorial Current, which flows westward and reaches these archipelagos with distinct characteristics (e.g., velocity) (Richardson and Walsh 1986). SPSP is also influenced by the Equatorial

Undercurrent, which flows in the opposite direction (eastward), approximately 80 m deep (Veleda et al. 2012). It decelerates when it reaches the SPSP archipelago, generating vortices on the east side and increasing the residence time of nutrients around the SPSP (Araujo and Cintra 2009), as commonly observed around seamounts (Mullineaux and Mills 1997).

Sampling

We captured 276 adult brown boobies on their nests by hand or hand net, and obtained the following body measurements of each individual: culmen length (exposed culmen), tarsus length (from middle of the midtarsal joint to the distal end of the tarsometatarsus), wing chord (carpal joint to the tip of the longest primary; unflattened wing), and body mass. We measured wing chord using a metal rule with stop (± 1 mm) and the remaining measurements using vernier calipers (± 0.01 mm). We obtained body mass using a digital balance and Pesola® spring scales, with precisions of 5 g and 10 g, respectively. We distinguished adults from juveniles by plumage coloration and gender by skin coloration differences around the eyes (Nelson 2005). Each bird received an individually numbered tarsal metal band to avoid resampling. Samplings were carried out both by the authors GTN and PLM. Data were obtained between August 2010 and December 2014 (Table 1).

Environmental data

We obtained sea surface and air temperature data for SPSP, FN, and Rocas from the nearest oceanographic buoys (Prediction and Research Moored Array in the Tropical Atlantic; PIRATA, located at 0°N, 35°W for SPSP and 8°S, 30°W for FN and Rocas)

(data available on <goosbrasil.org/pirata/>). Sea surface and air temperature data for the remaining sites were obtained from the fixed buoys nearest to each archipelago (Santa Catarina's buoy for Moleques [28.52°S, 47.37°W], Guanabara's buoy for Cagarras [22.91°S, 43.15°W], and Porto Seguro's buoy for Abrolhos [15.99°S, 37.95°W]), through the National Program of Buoys (data available on <goosbrasil.org/pnboia/>) (Fig. 1). We used the average temperatures between 1 January 2000 and 1 January 2015 recorded by the PIRATA buoys, and mean temperatures recorded between the launching date (March 2011, April 2012, and July 2012 for Moleques, Cagarras, and Abrolhos, respectively) and 21 March 2015, by the remaining buoys. We used chlorophyll α concentration estimated for summers (seasonal climatology) between 2002–2014, extracted from the Aqua MODIS, NASA/GSFC, using the Ocean Color Index (OCI) algorithm (data available on <modis.gsfc.nasa.gov/>). We downloaded the data series with a resolution of 4 km/pixel and calculated the average chlorophyll α value within a 40-km radius surrounding each colony, following previously published maximum foraging range of brown boobies (Weimerskirch et al. 2009, Soanes et al. 2015). Chlorophyll α concentration was used as a proxy for primary productivity (Huot et al. 2007).

Statistical analyses

To identify outliers in morphometric variables, we defined ± 2 standard deviations from the mean as a criterion, in order to rule out possible sampling errors while avoiding losing within-population variability. For body mass, we defined as outliers the values with ± 1 standard deviation, to avoid biases caused by the large individual daily fluctuation, before and after feeding. We calculated the correlation between allometric variables using Pearson's test. We assessed phenotypic differences among all populations with a one-way

MANOVA using the Wilk's lambda (λ), and a pairwise Hotelling's T-square as a post-hoc test. P-values were adjusted according to the Bonferroni procedure. We used residuals to test univariate normality using the Shapiro-Wilk's test, and multivariate normality with Mardia's test of skewness and kurtosis, Henze-Zirkler's, and Royston's tests, with the 'MVN' R package (Korkmaz et al. 2014). We assessed dissimilarity between populations using Euclidean distances based on a multivariate dataset (culmen, tarsus, wing, and body mass), with standardized values for all traits (subtracting the mean and dividing by the standard deviation). We generated dendrograms based on Euclidean distances by using the 'ggdendro' R package. We also used standardized values of culmen, wing, and tarsus to generate a body size index for each individual through a principal component analysis (adopting only the PC1 axis).

We used generalized linear models (GLM) with Gaussian family and identity link, to demonstrate which environmental variables better fitted the mass and body size data. We also tested correlations between all environmental variables (latitude, chlorophyll α, air and sea surface temperature) by using Spearman's ranking correlation with Bonferroni correction for multiple testing, in order to select uncorrelated variables for the regression model. High pairwise correlation values between morphometric variables (Pearson's r ranging from 0.49 to 0.74 for males and 0.61 to 0.65 for females; $df = 2$ and $p < 0.01$ for both genders in all pairwise tests; Supplementary material Appendix 1, Figure A1) allowed us to generate body size index adopting PC1, corresponding to 76.0% and 76.1% of the total variance for males and females, respectively. Culmen, tarsus, and wing lengths accounted for approximately 37.3%, 27.9%, and 34.7% for the male body size index, and 33.0%, 32.7%, and 34.1% for the female index, respectively. We did not include latitude in the regression models because this variable was significantly correlated to air temperature (Spearman's rho = 0.751; $df = 3$; $p < 0.01$). Therefore, regression models

were fitted only with air temperature (as the starting variable in a forward selection procedure), chlorophyll α and sea surface temperature as explanatory variables, and body size and mass as response variables. From this, we ran manual stepwise regressions, starting with air temperature (as postulated by Bergmann's rule) and adding the remaining variables through a forward procedure. We fitted models considering male body size index, male body mass, female body size index, and female body mass as distinct datasets. Moreover, we fitted models for these datasets in a scenario without SPSP, because it was an outlier to the clinal pattern (see 'Results'). We used Akaike's information criterion (AIC) as goodness-of-fit measure within datasets (the lower the better), and percentage of deviance explained to compare models fitted from distinct datasets (Burnham and Anderson 2002). All statistical analyses were performed with R software (R Core Team 2014).

Results

In total, we sampled 135 male and 141 female brown boobies. We detected female-biased dimorphism in all traits (culmen, tarsus, wing and body mass) in all the six sampled populations, females being 5.2% larger and 21.9% heavier than males (Table 2). For both females and males, the smallest individuals were those from FN and Rocas. Contrary to prediction, the colony closest to Equator (SPSP) had the largest and the heaviest males as well as the heaviest females (Fig. 2).

All allometric variables were found to have a multivariate normal distribution from all populations, and global multivariate population differences were found to be significant for both genders (MANOVA: females, Wilk's $\lambda = 0.061$, $df = 5$, $p < 0.001$; males, Wilk's $\lambda = 0.041$, $df = 5$, $p < 0.001$). Despite this, the post-hoc test showed no

significant difference between Cagarras and Moleques for both genders, and between Moleques and SPSP for females (Hotelling's T-square: $p < 0.001$; Table 3). We found the highest multivariate Euclidean distances between FN/Rocas and SPSP, the northernmost colonies (4.472 on average); and the lowest distances between Cagarras and Moleques (0.315 on average). We also found relatively low Euclidean distances among coastal colonies (0.912 on average), and between coastal colonies and SPSP (1.493 on average), demonstrating a phenotypic similarity between the colonies located in two geographical extremes of the area covered by the study (Table 3). Clustering using Euclidean distances based on multivariate data, generated dendrograms with two groups: a branch with the smallest and lightest individuals (FN/Rocas) and another branch with the remaining breeding sites (i.e. SPSP clustered with southern colonies; Fig. 3).

When using air temperature as the only explanatory variable in the regression models for all datasets, the explained percentage of deviance in body size and mass ranged from 0% to 2.3%, while in the scenario without SPSP the deviance explained of this model ranged from 50.6% to 72.9% (Fig. 4). In general, in both scenarios for all datasets, models improved (AIC decreasing and % deviance explained increasing) when we added chlorophyll α and SST (Supplementary material Appendix 2, Table A1). Finally, the model with air temperature and the interaction between chlorophyll α and sea surface temperature (air temperature + [chlorophyll α * SST]) showed the highest percentage of deviance explained (80.4%) and the lowest AIC for all datasets in both scenarios.

Discussion

Environment shapes phenotypes

In the context of Bergmann's rule, we demonstrated seabird phenotype variations along a tropical-subtropical gradient. However, in the present study, allometric variables did not present a direct correspondence with air temperature and latitude, and therefore did not fit to the Bergmann's rule (Bergmann 1847), since boobies at the colony located nearest to the Equator (SPSP) were phenotypically similar to the southernmost colonies. Nonetheless, in a scenario without SPSP, all allometric traits measured were correlated with air temperature, perfectly illustrating Carl Bergmann's hypothesis. Although air temperature did not correlate with allometrics when we consider SPSP, adding oceanographic variables (i.e. the interaction between chlorophyll α and sea surface temperature) substantially improved this relationship, suggesting influence of relevant additional factors in shaping size and body mass of marine organisms.

The negative correlation between body size and air temperature, observed in the scenario without SPSP, is purely empirical, regardless of the possibility that it could be physiologically explained. This finding is in close agreement with the heat conservation hypothesis, where the 'surface area to body volume' ratio is smaller in larger varieties of birds and mammals, thereby decreasing heat loss, which confers a clear advantage in colder regions (Rensch 1938). Air temperature is most likely not the only factor involved in shaping phenotypes, since it varies on a large spatial scale. While the complexity of population differentiation mechanisms and environmental variables can make it difficult to fully understand phenotypic diversification, it may also help to explain deviations from expected outcomes, such as those observed in brown boobies from SPSP.

Although the SPSP archipelago is on the Equator and its phytoplankton community is characteristic of oligotrophic tropical waters (Tiburcio et al. 2011), the interaction of the Equatorial Undercurrent with the archipelago topography slows the movement of the current at a local level and generates vortices, which increase the mixing of the water column (i.e. disrupting the surface thermocline) and the residence time of waters around the SPSP (Araujo and Cintra 2009). This process allows for the retention of nutrients from allochthonous or autochthonous sources (e.g., seabird guano), making the biomass and zooplankton productivity in the vicinity of SPSP similar to those observed around seamounts (Mullineaux and Mills 1997, Melo et al. 2014). A pattern of higher productivity and presence of relatively colder waters closer to the surface is more pronounced around SPSP than nearby tropical islands sampled for this study, i.e. FN (Souza et al. 2013) and Rocas (Jales et al. 2015). In addition, ichthyoplankton are more abundant around the SPSP than around FN, mainly because the former is a spawning area for Exocoetidae flyingfishes, the preferential food item of brown boobies (Lessa et al. 1999, Mancini and Bugoni 2014). Although spatially very restricted, local productivity around SPSP is increased by small-scale oceanographic dynamics, making it an oasis in a tropical oligotrophic area and sustaining migratory and resident endemic fishes, as well as fisheries production (Vaske-Jr. et al. 2005, 2008).

In general, productive areas in the ocean favor larger organisms, decreasing the energy path along the trophic web, so that energy transfer from the base to the top is optimized (Pinet 2009). We found larger boobies in coastal colonies and, therefore, near to more productive waters (Mann and Lazier 2006). Accordingly, smaller and lighter boobies were found at FN and Rocas, which are outside the Brazilian continental shelf and are influenced mainly by the oligotrophic waters of the South Equatorial Current (Garrison 2010). Therefore, chlorophyll α, here used as a proxy for primary productivity,

and sea surface temperature, although they show no correlation with latitude and air temperature, sheds light on spatial small-scale processes shaping phenotypes. In this context, allometric variation in marine organisms could be better explained when combining air temperature with small-scale oceanographic parameters, as primary productivity and sea surface temperature.

Although the best model explained over 80% of the deviance for all datasets, the sharp deviation from the clinal pattern observed in SPSP could be enhanced by the relatively restricted area for breeding. The SPSP colony holds approximately 200 nests (Mancini et al. 2016) and a suitable nesting area of only about 200 m2, where average distance between nests is 1 m and average nest diameter of 0.36 m, while the Rocas colony have an average distance of 11.2 m between nests and average nest diameter of 0.44 m (Kohlrausch 2003). The high density of nests at SPSP could favor larger and heavier boobies when competing for nesting areas. Furthermore, the crowded nesting colony at SPSP has also been postulated to alter behavior, resulting in frequent cannibalism in this colony, but not elsewhere (Neves et al. 2015). Thus, the restricted spatial area for nesting, along with environmental variables, could intensify phenotypic differences between boobies from SPSP in comparison with those from the nearby FN and Rocas, representing an example of ecology driving evolution and, consequently, isolation by environment as a mechanism of population divergence.

Phenotypic diversity

Our findings demonstrated a high phenotypic diversity of brown boobies along the Southwest Atlantic Ocean colonies. Coastal colonies were found to be similar, presenting boobies with intermediate body size and mass. Rocas and FN also proved to be quite

similar to each other, consistent with limited distance between colonies (~150 km) and similar environmental constraints. Dietary differences between brown booby populations have been observed through regurgitates and stable isotopes (δ13C and δ15N), as well as differences in isotopic niches among brown boobies from SPSP, Rocas, FN, and Abrolhos (Mancini et al. 2014). The wider isotopic niche observed from Abrolhos was thought to be related to higher baseline isotopic values and/or to a more generalist diet, as Abrolhos was the colony closest to the coast, where prey diversity could be also influenced by discards from demersal fisheries. The dependence of brown boobies on discards from demersal fisheries has been reported in other coastal colonies along the Brazilian continental shelf (Coelho et al. 2004, Krul 2004, Branco et al. 2005). Waters around coastal archipelagos have higher productivity than oceanic environments and, therefore, the isotopic range of brown boobies in Abrolhos could be similar to other coastal sites. This could explain the phenotypic similarity among brown boobies sampled from coastal colonies (Abrolhos, Cagarras, and Moleques). Moreover, long-term climatic stability tends to promote genetic differentiation even among conspecific populations (Carnaval et al. 2009, Rodríguez-Robles et al. 2010). In this context, relatively stable climatic conditions of tropical regions (Evans et al. 2002, Tierney et al. 2015) could enhance population structure observed in our study, by keeping environmental conditions around colonies stable along an evolutionary time-scale and promoting genetic divergence according to the structuring based on phenotypes.

On the other hand, we observed an unexpected phenotypic similarity between SPSP and all other coastal colonies, particularly the southernmost one at Moleques, which are located in the extremes of latitude of our study area. Regarding distribution around colonies, tracking brown boobies during foraging trips throughout the breeding period at SPSP (GT Nunes, unpub. data) has revealed a mean foraging trip duration of only ~1 h

for both genders, and a mean maximum distance from colony of ~9 km. These values are much lower than those reported for other populations elsewhere (~2.5 h and ~28 km in the Gulf of California, Weimerskirch et al. 2009; ~5 h and ~ 38 km in the Caribbean, Soanes et al. 2015). Although there is no information on the distribution of these individuals during the non-breeding period, SPSP population shows constant adult abundance and breeding along the whole year (Barbosa-Filho and Vooren 2010), which, together with the brief trips observed, suggest that obtaining food close to SPSP may not be difficult. In this case, dispersal could not be beneficial, increasing philopatry of this population and strengthening the hypothesis of adaptive convergence in relation to the coastal colonies.

Divergences among brown booby colonies have already been observed in the Atlantic Ocean, although using a purely genotypic approach (Morris-Pocock et al. 2011). These authors revealed two clusters in the Atlantic Ocean (Ascension/Cape Verde and the Caribbean Isla Monito), suggesting natal philopatry as the mechanism of population differentiation. Additional mechanisms, such as nonbreeding distribution patterns, could also contribute to population isolation (Friesen 2005), although it remains unknown for brown boobies. Here, we proposed that the level of natal philopatry can be a consequence of distinct pressures (e.g., environmental variables) and it could be understood as dispersal cost, which is determined by the degree of adaptive diversification generated from ecological differences between colonies (Weatherhead and Forbes 1994). In this context, environmental variables with oceanographic parameters (both large and small spatial scales) should be a primer in explaining phenotypic diversity in the marine realm, since it also enables monitoring climate change effects on marine organisms considering intraspecific diversity (Quillfeldt and Masello 2013).

Tropical marine environments tend to be stable throughout the year, but an increase in sea surface temperature during the last 100 years has been observed in tropical oceans (Hoegh-Guldberg 1999). Looking forward, the most extreme Representative Concentration Pathway (greenhouse gas concentration trajectory RCP 8.5) predicts mean global surface temperature increasing by 7–8° C between now and the year 2300 (IPCC 2014). This could have several implications for ecological processes, such as alterations in atmospheric and oceanic circulation. This phenomenon has already been observed in the Bering Sea, where the decreased recruitment of juvenile walleye pollock (Theragra chalcogramma), a local key fish species, has affected other fish species, marine mammals, and seabirds (Wespestad et al. 2000). In this context, brown booby populations could be affected in distinct ways, as breeding sites with low prey diversity (i.e. dependence on a few key prey species) could be impacted earlier by rapid climate change.

Conclusions

This study demonstrates that Bergmann's rule does not apply to brown boobies nesting on islands along the Brazilian coast. The observed deviation sheds light on the role of other selection pressures, such as small-scale oceanographic processes, in shaping body size along a broad latitudinal gradient in the marine realm. Therefore, studies on phenotypic diversity and conformance with Bergmann's rule in seabirds (or even in other marine animals) should also consider oceanographic parameters as potential direct or indirect mechanisms of population differentiation, so these local conditions could be drivers for intraspecific phenotypic diversification, although additional pressures (e.g., founder effect, nest density, and behavioral aspects) could not be ruled out to explain phenotypic diversity. Finally, if phenotypes mirror genotypes in brown boobies from

Brazil, we suggest the existence of three molecularly supported clusters (SPSP, Rocas/FN, and Abrolhos/Cagarras/Moleques), with the similarity between SPSP and Abrolhos/Cagarras/Moleques owing to a case of adaptive convergence driven by environmental pressures. Further testing will be required to elucidate gene flow between the brown boobies along the Brazilian coast.

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Table 1. Approximate distances from the coast (km), abundance, and location of the six colonies of brown boobies sampled.

Archipelago	Abundance (number of individuals)	Location	Sampling events
Saint Peter and Saint Paul	600[1]	0°55'51"N; 29°20'45"W	May, Jun, Jul, Aug 2011–2015
Fernando de Noronha	870[2]	3°51'15"S; 32°25'44"W	Mar, Apr 2011
Rocas Atoll	140[3]	3°52'30"S; 33°48'20"W	Sep 2010, Feb 2012
Abrolhos	550[1]	17°57'46"S; 38°42'10"W	Feb, Aug 2011
Cagarras	2500[4]	23°01'35"S; 43°11'33"W	Dec 2014
Moleques do Sul	1700[5]	27°50'46"S; 48°25'53"W	Feb 2014

[1] Mancini et al. (2016); [2] Antas (1991); [3] Schulz-Neto (2004); [4] Cunha et al. (2013); [5] Branco et al. (2010)

Table 2. Means ± standard deviations of culmen, wing, tarsus, and body mass measurements from six colonies of brown boobies (*Sula leucogaster*) sampled in Brazil, from north to south. SPSP = Saint Peter and Saint Paul archipelago; FN = Fernando de Noronha archipelago; Rocas = Rocas Atoll; Moleques = Moleques do Sul.

Locations and sample size (n)	Culmen (mm)		Tarsus (mm)		Wing (mm)		Body mass (g)	
	♂	♀	♂	♀	♂	♀	♂	♀
SPSP (124)	103.04 ± 2.03	106.93 ± 2.63	48.13 ± 3.22	50.51 ± 1.75	410.92 ± 6.41	425.23 ± 6.14	1311.27 ± 80.11	1620.78 ± 87.92
FN (35)	92.45 ± 2.72	96.54 ± 3.03	42.49 ± 1.41	44.43 ± 2.14	382.76 ± 8.03	399.33 ± 11.13	867.05 ± 57.96	1061.33 ± 115.50
Rocas (31)	90.63 ± 2.31	95.09 ± 2.38	44.98 ± 1.69	47.11 ± 1.41	388.50 ± 4.05	403.53 ± 9.69	934.16 ± 65.01	1137.69 ± 85.16
Abrolhos (40)	99.91 ± 2.13	102.08 ± 2.14	46.33 ± 1.63	49.14 ± 1.75	404.38 ± 8.50	425.94 ± 9.18	1082.22 ± 98.49	1305.29 ± 179.30
Cagarras (28)	95.79 ± 1.05	103.77 ± 2.82	47.47 ± 0.95	51.01 ± 0.87	402.09 ± 6.64	426.01 ± 9.11	1210.45 ± 53.59	1465.45 ± 62.98
Moleques (18)	97.15 ± 2.21	103.68 ± 2.94	47.58 ± 1.23	51.32 ± 0.98	404.28 ± 4.46	426.14 ± 8.35	1235.71 ± 56.52	1511.42 ± 69.38

Table 3. Euclidean distances based on a multivariate dataset of allometric variables (culmen, tarsus, wing, and body mass) between all brown booby colonies sampled along the Brazilian coast. Females are represented by values above the diagonal, and males by values below the diagonal. Bold values represent Hotelling's T-squared significant differences, with adjusted p-values based on the Bonferroni correction for multiple comparisons ($p < 0.01$).

SPSP	**4.567**	**3.998**	**1.851**	**1.005**	0.919
5.044	FN	**1.150**	**3.188**	**4.032**	**4.200**
4.279	**1.399**	Rocas	**2.568**	**3.321**	**3.466**
1.794	**3.374**	**2.706**	Abrolhos	**1.070**	**1.286**
1.889	**3.604**	**2.578**	**1.285**	Cagarras	0.240
1.504	**3.886**	**2.923**	**1.205**	0.390	Moleques

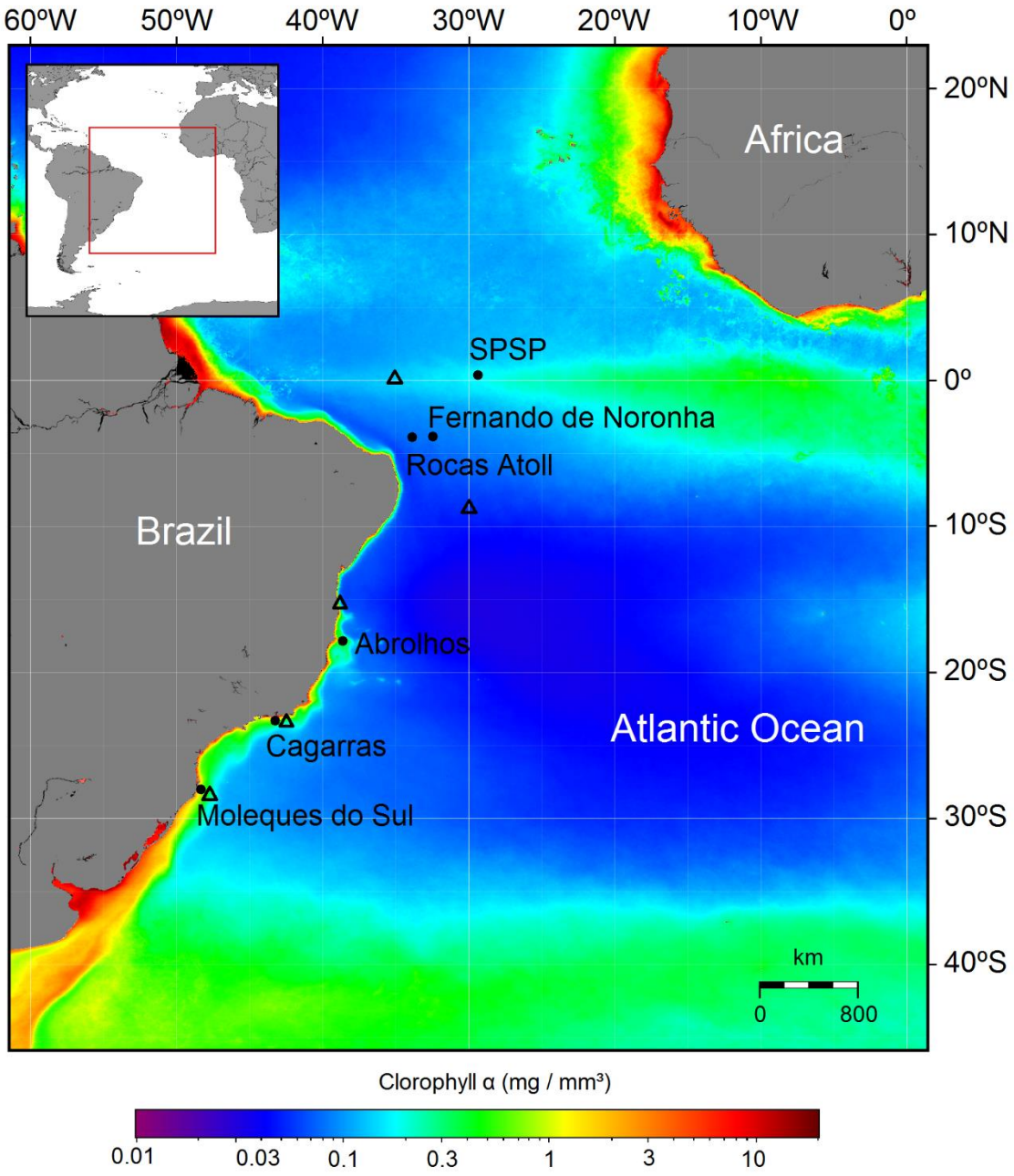

Figure 1. Geographic locations of the six brown booby (Sula leucogaster) colonies superimposed on data representing the average chlorophyll α concentration (mg/m2) layer during the past 15 years. Archipelagos are identified by closed circles, while oceanographic buoys are identified by open triangles. Data was extracted and modified from <oceancolor.gsfc.nasa.gov/SeaWiFS/>. SPSP = Saint Peter and Saint Paul archipelago.

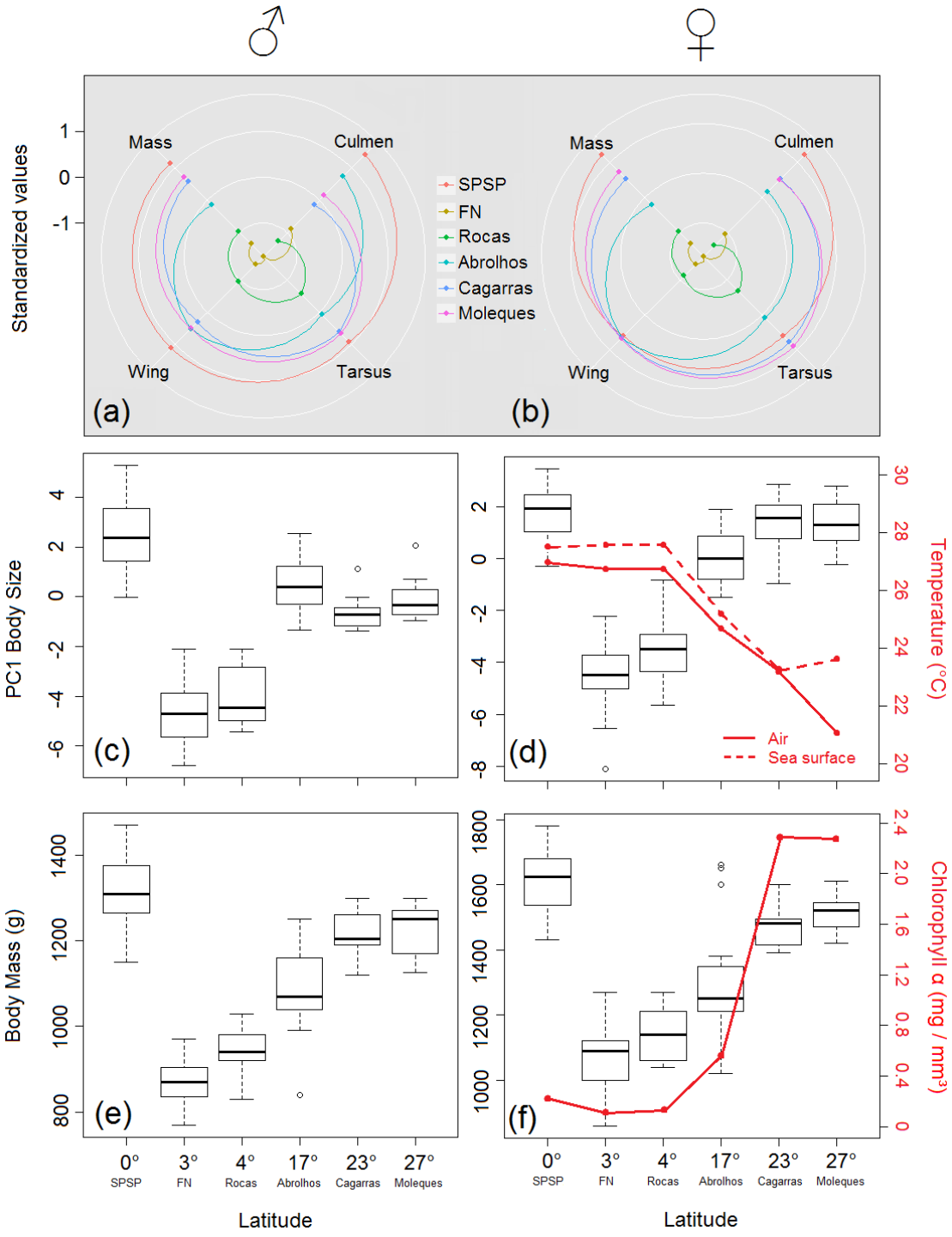

Figure 2. (a, b) Radial plots comparing standardized values (subtracting the mean and dividing by the standard deviation) of culmen, wing chord, tarsus, and body mass among all sampled colonies for males (left) and females (right). Radial lines correspond to quantitative variables, with values decreasing toward the center. Each color corresponds to one archipelago, and each point on radial lines corresponds to the population mean (the

ellipse '0' corresponds to the mean for each variable). (c, d) Clinal variation of the intraspecific variation in the body size index (first principal component from culmen, tarsus, and wing measurements) within archipelagos; and (e, f) body mass values distributed on a latitudinal gradient. Air and sea surface temperatures (in red) are reported in (d) and chlorophyll α in (f). Males and females are represented by left and right panels, respectively.

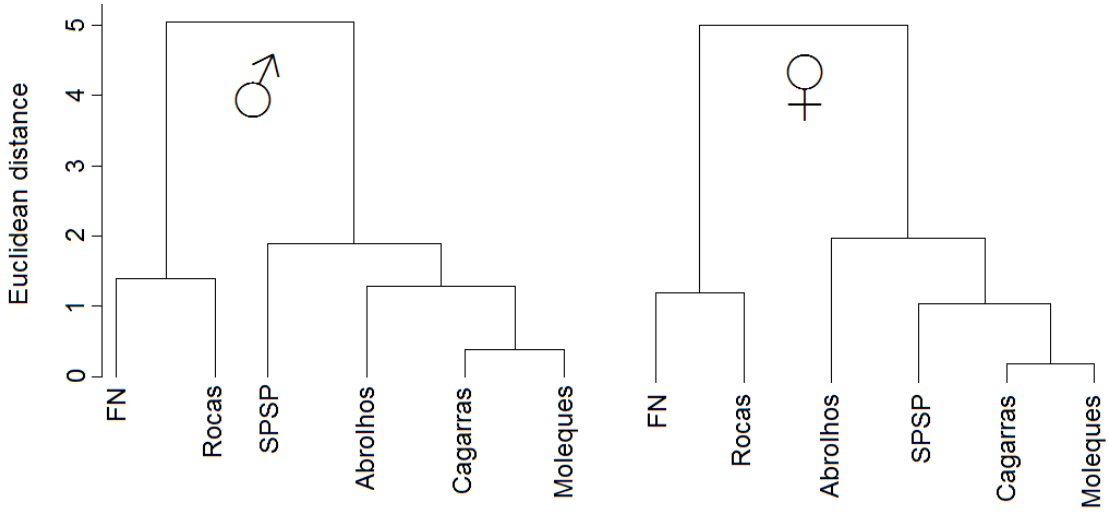

Figure 3. Dendrograms based on variation of the multivariate Euclidean distances (y-axis) from standardized values of culmen, tarsus, wing and body mass for males (left) and females (right). SPSP = Saint Peter and Saint Paul archipelago; FN = Fernando de Noronha archipelago; Rocas = Rocas Atoll; Moleques = Moleques do Sul.

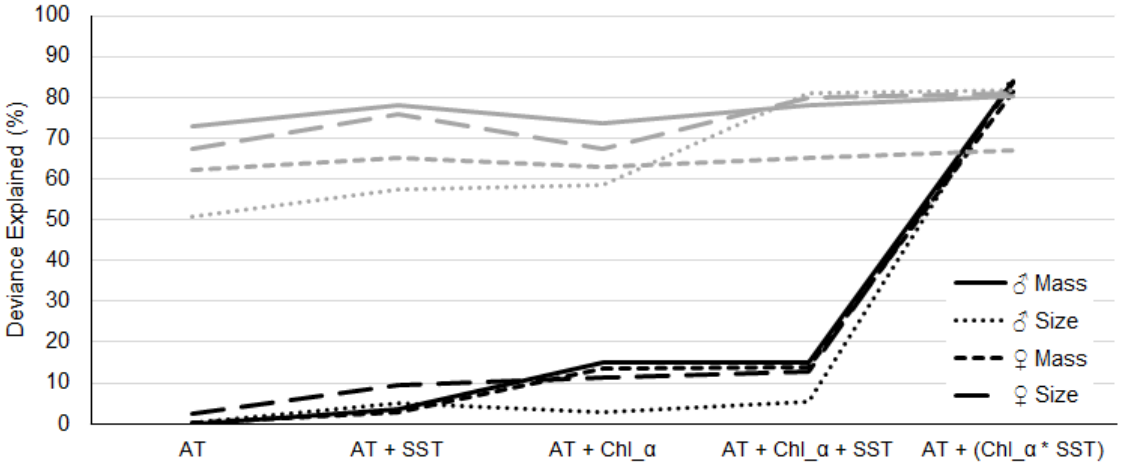

Figure 4. Gaussian regression models fitted with body mass and body size (culmen, tarsus, and wing integrated in a body size index) for both males and females as response variables, and air temperature (AT), sea surface temperature (SST), and chlorophyll α (Chl_α) as explanatory variables. Black lines refer to the scenario with the Saint Peter and Saint Paul archipelago, and grey lines refer to the scenario without the Saint Peter and Saint Paul archipelago.

Supplementary material

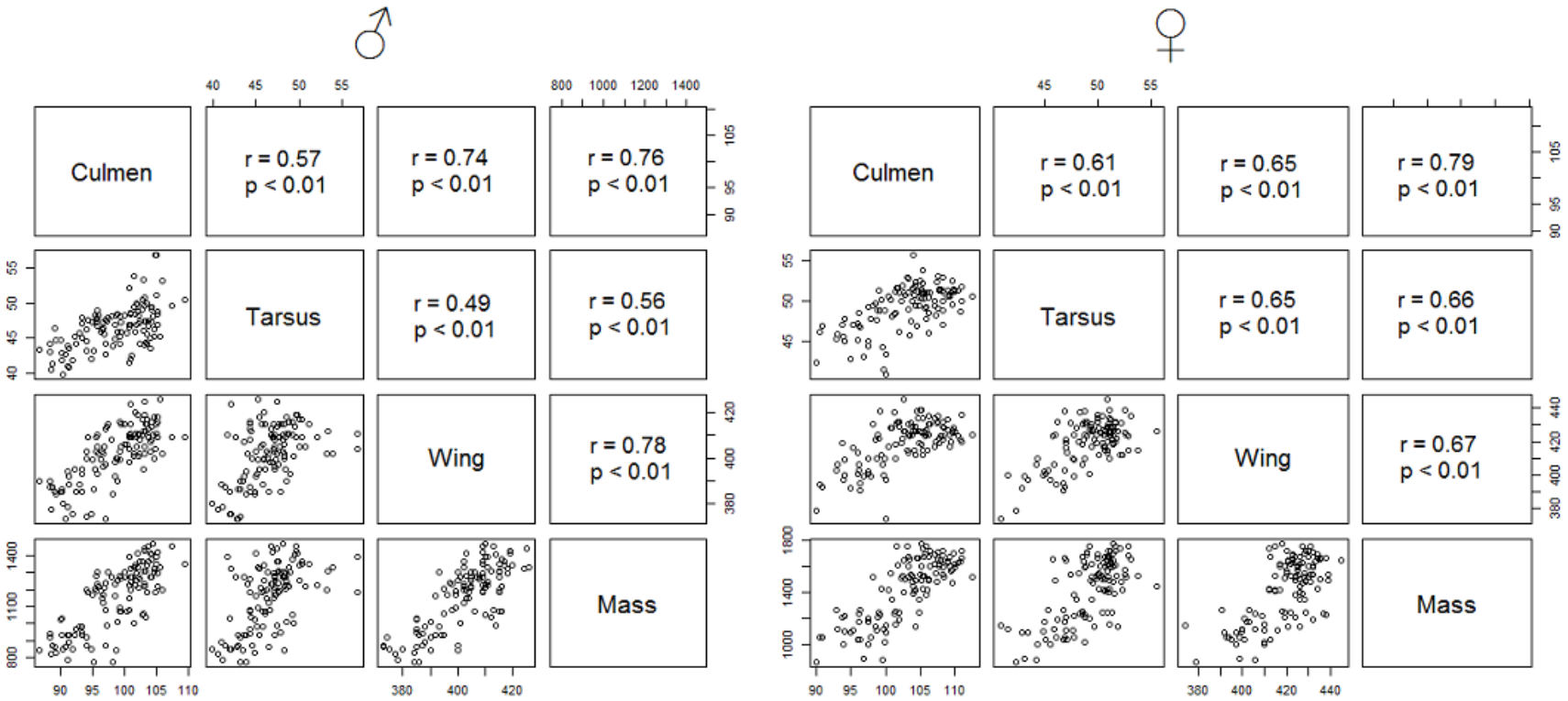

Figure A1. Scatterplot matrices of paired Pearson's correlations among culmen, wing chord, tarsus, and body mass for males (left) and females (right).

Table A1. Percentage of deviance explained (% DE) and Akaike Information Criterion (AIC) for each regression model fitted with datasets from male body mass, male body size index, female body mass, and female body size index in the scenarios with and without the Saint Peter and Saint Paul (SPSP) archipelago. Bold values are representing the highest % DE and the lowest AIC for each dataset. AT = air temperature; SST = sea surface temperature; Chl_α = chlorophyll α.

	WITH SPSP								WITHOUT SPSP							
	♂				♀				♂				♀			
Models	Mass		Size		Mass		Size		Mass		Size		Mass		Size	
	% DE	AIC	% DE	AIC	% DE	AIC	% DE	AIC	% DE	AIC	% DE	AIC	% DE	AIC	% DE	AIC
AT	0.0	565.81	0.4	586.97	0.1	529.72	2.3	550.80	72.9	211.11	50.6	262.10	62.0	212.68	67.5	240.81
AT + SST	3.7	563.42	4.9	583.55	2.7	528.69	9.4	544.24	78.0	199.58	57.5	254.27	**65.3**	**208.97**	75.8	224.24
AT + Chl_α	14.9	549.06	2.9	586.04	13.6	515.21	11.3	541.83	73.6	211.49	58.5	252.67	62.9	213.16	67.5	242.81
AT + Chl_α + SST	14.9	551.06	5.3	585.10	13.8	516.90	12.8	541.94	78.1	201.14	80.9	204.18	65.3	210.95	79.7	214.99
AT + (Chl_α * SST)	**83.9**	**359.80**	**85.2**	**371.77**	**81.4**	**344.24**	**83.4**	**354.52**	**80.1**	**196.97**	**81.6**	**203.87**	66.8	210.10	**81.1**	**212.61**

Anexo 2

Running headline: Isolation by environment in seabirds

Seascape and local adaptation driving population isolation in seabirds

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Summary

1. Environmental heterogeneity tends to increase biological diversity by promoting population isolation. Geographical distance is an important driver of population isolation, such that genetic differentiation among populations increases as geographical distance between populations increases (i.e. Isolation by Distance - IBD).

2. However, seascape has been shown to play a major role in the evolutionary processes of marine animals, even contributing to the differentiation of populations of highly mobile, closely-related organisms (i.e. Isolation by Environment - IBE).

3. In this study, seabirds breeding across a heterogeneous seascape in the southwestern Atlantic Ocean were used to test the role of IBD and IBE models in promoting intraspecific diversity. For this work, 119 brown boobies *Sula leucogaster* breeding in six colonies along a latitudinal gradient (0–27°S) were sampled. Genetic diversity, population structure, and dispersal rates among colonies were assessed through nine microsatellite loci. Geographical distances and empirical data sets of physical parameters (i.e. air temperature, sea surface temperature, and chlorophyll α concentration) and colony-specific ecological parameters (i.e. colony density and isotopic niche width) were used to test the role of IBD and IBE in genetic-based clustering.

4. Genetic isolation of a remote small colony was associated with specialization on local resources, which was suggested to promote selection against migrants. Clustering of the remaining colonies was explained by seascape differences between neritic and oceanic environments.

5. Seabirds can easily overcome geographical distances separating populations. However, their dispersal ability seems to be lower than their mobility, such that population isolation can be better explained by environmental heterogeneity (IBE) than by geographical

distance (IBD). In this context, seabirds emerge as promising models for understanding how wild populations will respond to alterations in environmental parameters caused by climate change.

Key-words: Atlantic Ocean, brown boobies, evolutionary ecology, gene flow, isolation by distance, isolation by environment, marine ecology, microsatellite, oceanographic dynamics, *Sula leucogaster*.

Introduction

Understanding how biodiversity correlates with environmental features represents a baseline for evolutionary ecologists studying all kinds of organisms in any ecosystem (Richardson *et al.* 2014). Identifying associations of biological diversity with selection pressures reveals the microevolutionary processes accounting for existing forms and enables one to predict how wild populations will respond to climate changes (Hoffmann & Sgrò 2011). In general, ecogeographical rules (e.g. Bergmann's rule; Bergmann 1847) can explain phenotypic variation of a wide range of species, but known exceptions shed light on the complexity of mechanisms shaping biodiversity (e.g. Fisher, Frank & Leggett 2010; Berke *et al.* 2013; Nunes, Mancini & Bugoni 2017). Mayr (1956) highlighted this conflict and suggested that, "The emphasis of research should be shifted to a study of the exceptions." Therefore, studies addressing the association of intraspecific diversity and landscape heterogeneity can provide a better understanding of the drivers of population isolation and how evolutionary processes can be used to predict impacts on biodiversity under rapid climate change (Orsini *et al.* 2013).

Early studies proposed that genetic differentiation among populations can increase as geographical distance increases (e.g. Wright 1943). Under the Isolation by Distance model (IBD), genetic drift would cause population differentiation in the absence of selection and reduce dispersal rates with increasing geographical distance (Wright 1943, 1946). The IBD model has been demonstrated for large spatial scales and applied to organisms with low dispersal capacity, resulting in a pattern of spatial autocorrelation in the distribution of genetic variation (Meirmans 2012), which is mostly applied to plants (Vekemans & Hardy 2004) and sessile invertebrates (Maier *et al.* 2005). When observed

in vertebrates, it has been attributed to stochastic (Pogson *et al.* 2001) or deterministic conservation issues (Hernández *et al.* 2016).

Alternatively, it has been shown that not only geographical distances influence population structure, but that local adaptation can also reduce gene flow and be a major driver of population differentiation (Sexton, Hangartner & Hoffmann 2014). The Isolation by Environment model (IBE) may be defined "as a pattern in which genetic differentiation increases with environmental differences, independent of geographical distance" (Wang & Bradburd 2014). Therefore, landscape heterogeneity is a basic assumption of the IBE model and some ecological processes are known to contribute to population isolation, such as biased dispersal (Edelaar & Bolnick 2012), and natural and sexual selection against migrants due to local adaptation (Hendry 2004). In this case, fine-scale environmental variations are sufficient to act as barriers between populations, such as differences in size of seeds for passerines (Ryan *et al.* 2007), forest types for epiphytic orchids (Mallet *et al.* 2014), and topography for grasshoppers (Noguerales, Cordero & Ortega 2016).

For marine organisms, seascape features also work as barriers for population isolation, such as sea surface temperature for dolphins (Amaral *et al.* 2012), and bathymetry for fishes (Hyde *et al.* 2008) and giant kelps (Johansson *et al.* 2015). Forces driving spatial genetics (by promoting or disrupting gene flow) in marine populations can be even more complex by including population-specific parameters, such as population density and trophic relationships (Selkoe *et al.* 2016). In this context, local pressures can induce local adaptation and change gene frequencies in a population, which, in turn, can affect population dynamics (Saccheri & Hanski 2006).

Over the last decade, landscape genetics has been matching spatial ecology to disentangle these eco-evolutionary processes and demonstrate IBE (Manel & Holderegger 2013). Nevertheless, assessments of spatial genetics in marine populations are still scarce when compared to terrestrial populations, presumably due to the inherent difficulty in characterizing marine habitats and populations (Selkoe *et al.* 2016). Given this, seabirds arise as interesting models for studying relationships between genetic diversity and spatial ecology, as breeding activities mostly take place on islands, and although they can travel thousands of kilometres in a few days (e.g. Fijn *et al.* 2013), the group is characterized by its high philopatry (Schreiber & Burger 2001). Furthermore, seabirds are widely distributed, making it possible to compare populations across environmental gradients, which is the case of the brown booby, *Sula leucogaster* (Boddaert, 1783), a strictly marine bird living in tropical and subtropical latitudes in all ocean basins (Carboneras 1992). Brown boobies forage by plunge diving around their colonies during the breeding period and are believed to not perform true migration (predictable movements) during wintering periods, remaining nearby their colonies throughout the year (Nelson 2005).

Intraspecific genetic diversity is high in brown boobies and distinct populations can be found even within the Atlantic Ocean (Morris-Pocock, Anderson & Friesen 2011). In the southwestern Atlantic Ocean, there is also evidence of phenotypic population differentiation in brown boobies driven by multi-scale environmental parameters (Nunes, Mancini & Bugoni 2017), along with differences in trophic niche width (Mancini, Hobson & Bugoni 2014) and population size (Mancini, Serafini & Bugoni 2016). Although geographically near to each other, brown booby populations in the southwestern Atlantic

Ocean seem to be shaped by local pressures, which have been suggested as important triggers for speciation in seabirds (Friesen 2015).

Therefore, the role of environmental heterogeneity in influencing population structure of a highly mobile top predator was assessed in this study. To do this, microsatellites were used to estimate the contemporary genetic diversity of brown boobies across six colonies, along with a data set of physical and colony-specific parameters, to investigate how the environment can promote or disrupt gene flow. From this, the following issues were assessed: (1) population differentiation and structure based on genotypes; (2) asymmetrical dispersal among colonies; and (3) the role of IBD and IBE in population isolation of brown boobies in the southwestern Atlantic Ocean.

Materials and methods

SAMPLING AND STUDY AREA

Along a latitudinal gradient from 0° to 27°S in the southwestern Atlantic Ocean, sampling was carried out between 2011 and 2014 in six archipelagos: Moleques do Sul, Cagarras, Abrolhos, Rocas Atoll, Fernando de Noronha, and Saint Peter and Saint Paul, hereinafter referred to as, Moleques, Cagarras, Abrolhos, Rocas, FN, and SPSP, respectively. Air temperature (AT) tends to follow a clinal pattern in relation to the latitude gradient, but sea surface temperature (SST) and primary productivity are influenced mainly by the oceanographic dynamics around archipelagos. Nunes, Mancini & Bugoni (2017) provided further details on multi-scale environmental parameters for these archipelagos.

Sampling was concentrated on breeding adults, distinguished from juveniles by plumage coloration (Nelson 2005). Boobies were captured on nests by hand or using hand

nets, and blood samples (~200 µl) were obtained by puncturing the tarsal vein with a sterile needle, and stored in microtubes containing 70% ethanol. Individuals were identified with metal bands to avoid resampling, and released on the nests after handling.

GENETIC DIVERSITY AND STRUCTURE

DNA was extracted from blood samples using standard protocols. All samples were amplified at nine microsatellite loci with previously described primers (Taylor *et al.* 2010). M13(-21) tail was incorporated into each forward primer at the 5' end (Schuelke 2000) (see Appendix S1 for details on DNA extraction and PCR conditions).

Evidence for null alleles, large allele dropouts, and scoring errors due to stuttering were checked using MICRO-CHECKER v2.2.3 (van Oosterhout *et al.* 2004). Genotypic linkage disequilibrium for each pair of loci in each population and across all populations was assessed through Fisher's exact tests in GENEPOP v4.4 (Rousset 2008). Observed and expected heterozygosities (H_O and H_E), deviations from Hardy-Weinberg equilibrium (HWE; Nei 1978), and allelic richness (A) were calculated using ARLEQUIN v3.5 (Excoffier & Lischer 2010). Bonferroni correction was used as a control for multiple tests (Legendre & Legendre 2012). Allele frequencies were calculated in order to assess the proportion of rare alleles for each population (Luikart *et al.* 1998). Wilcoxon signed-rank test, as implemented in BOTTLENECK v1.2.02, was used to test for heterozygosity excess (Cornuet & Luikart 1996). Computations assumed a two-phase mutation model (TPM; Di Rienzo *et al.* 1994) with the variance among multiple steps equal to 12 and the proportion of multi-step mutations equal to 0.22 (Peery *et al.* 2012). Finally, the percentage of polymorphic loci and the inbreeding coefficient F_{IS} (Weir & Cockerham 1984) were calculated for each population in GENALEX v6.5 (Peakall & Smouse 2012).

Nei's F_{ST} (Nei 1977) was used to measure genetic distance between the six populations, which was calculated in GENALEX v6.5. Fisher's exact test with sequential Bonferroni correction (Legendre & Legendre 2012) was applied to detect intraspecific genotypic differences in GENEPOP v4.4. A phylogenetic tree was built using the unweighted pair-group method with arithmetic mean (UPGMA) (Sneath & Sokal 1973), as implemented in the software POPTREE2 (Takezaki, Nei & Tamura 2010). Principal coordinate analysis (PCoA) was also carried out in GENALEX v6.5 with standardized data in order to visually identify similarities and groupings among colonies. Additionally, Bayesian clustering implemented in the software STRUCTURE v2.3.4 was performed to determine the most plausible number of clusters (K) (Pritchard, Stephens & Donnelly 2000). Numbers of K from 1 to 10 were tested by conducting 20 independent runs for each K with a burn-in period of 100,000 steps and 1,000,000 MCMC repetitions. The ad hoc ΔK (Evanno, Regnaut & Goudet 2005) was used to detect the best K in STRUCTURE HARVESTER Web v0.6.94 (Earl & vonHoldt 2012). The 20 independent runs for the best K were merged using CLUMPP v1.1.2 (Jakobsson & Rosenberg 2007) and a bar plot was generated using DISTRUCT v1.1 (Rosenberg 2004). Finally, a Bayesian inference of recent bidirectional gene flow was performed using BAYESASS v1.3 (Wilson & Rannala 2003).

ISOLATION BY DISTANCE

Geographical coordinates of each colony were used to calculate pairwise geographical distances (km; hereinafter, “GeoDis”). Using this information, the IBD was tested through a Mantel test by assessing correspondence between geographical and genetic matrices, which were log-transformed (1 + log(distance)) and linearized (Slatkin 1995),

respectively. A matrix of Nei's pairwise F_{ST} was built with the HIERFSTAT package in the R software (Goudet & Jombart 2015; R Core Team 2016). A Mantel test was performed with Pearson's correlation and *P*-values were calculated using 5,000 permutations in the VEGAN package (Oksanen *et al.* 2016) in the R software.

ISOLATION BY ENVIRONMENT

Distance from the coast (km; "DisCoast") was obtained for each archipelago in Google Earth Pro by measuring the shortest straight-line distance to the mainland. Population density was calculated as individuals/km^2, hereinafter referred to as "Density" (see Appendix S1 for details on calculations). AT (°C), SST (°C), and chorophyll α concentration (mg.mm^{-3}; "Chlα") for each sampling site were obtained from Nunes, Mancini & Bugoni (2017).

Isotopic niche width was estimated for each population with carbon and nitrogen isotopic ratios from whole blood, obtained from Mancini, Hobson, & Bugoni (2014) and from samples taken and processed by the authors (see Appendix S1 for details on samples and isotopic data processing). Bayesian ellipse areas were calculated in the package SIBER (Jackson *et al.* 2011), and isotopic niche width was defined as the standard ellipse areas adjusted for small sample sizes (‰^{2}; "SEAc").

Pairwise Pearson's correlations were calculated among AT, SST, Chlα, DisCoast, Density, and SEAc using sequential Bonferroni (Legendre & Legendre 2012). Mantel tests were performed between genetic matrix and distance matrices of each environmental variable, which were calculated with the Mahalanobis dissimilarity index in the VEGAN package.

Two distinct approaches were applied to identify the best combination of environmental variables to explain genetic structure of brown boobies. The first used hierarchical Bayesian generalized linear models (GLM) implemented in the software GESTE 2.0, so that environmental features were used as independent variables to explain population-specific F_{ST}'s (Foll & Gaggiotti 2006). To fit GLM's, 10,000 MCMC iterations with 100 iterations between two samples were run to reduce autocorrelation. The 95% highest probability density interval (HPDI) was used to measure the degree of uncertainty of the estimations, and the best model was that with the highest posterior probability. The second approach used was a Redundancy Analysis (RDA), a multivariate approach based on the assumption that the relationship between the unconstrained matrix (allele frequencies) and constrained matrix (environmental data) is linear. The VEGAN package was used to identify the contribution of each environmental variable on genetic variation by running the *ordistep* function through a backward stepwise procedure and using Akaike's Information Criteria (AIC) to select the best model. *P*-values were calculated based on 10,000 permutations.

Results

A total of 119 individuals were sampled, ranging from 18 individuals in Moleques to 24 in SPSP. MICRO-CHECKER results demonstrated no significant presence of null alleles, scoring errors, or allelic dropout. Only 2 out of 54 loci (6 populations × 9 loci) deviated from HWE (*P*-value < 0.05), and 3 out of 216 loci pairs (6 populations × 36 loci pairs per population) deviated from linkage equilibrium (*P*-value < 0.05). No linkage disequilibrium was detected when comparing loci pairs across all populations. The nine

loci were polymorphic, with 2.6 alleles on average, but boobies from SPSP had low polymorphism (44.4%) when compared to those from Rocas/Abrolhos/Cagarras (88.9%) and those from FN/Moleques (100%). SPSP boobies also had the lowest H_O and allelic richness (Table 1), and a low percentage of rare alleles (~8%). The inbreeding coefficient (F_{IS}) was close to 0 for all populations, but the TPM model indicated significant (*P*-value = 0.005) heterozygosity excess for Rocas boobies, while it was marginally non-significant (*P*-value = 0.06) for SPSP boobies, with a mode-shift distortion in both colonies (Fig. S1).

The PCoA plot was built from coordinates 1 and 2, which explained 73.9% and 18.0% of the genetic variance, respectively. Coordinate 1 separated the SPSP colony from the remaining colonies, while Coordinate 2 identified differences between FN-Rocas and Moleques-Cagarras-Abrolhos, hereinafter referred to as 'coastal colonies', since they are on the continental shelf '(Fig. S2). Therefore, bar plots from the STRUCTURE results were built considering both K = 2 and K = 3 (Fig. 1B, see Fig. S3 for results from the STRUCTURE HARVESTER). The UPGMA tree suggested that these may be considered as two clusters, by slightly splitting FN/Rocas from coastal colonies (Fig. 1C). The lowest F_{ST} values were observed for the Cagarras-Moleques and Rocas-FN colonies, which also presented no significant genotypic differences (Table 2). The highest rates of gene flow were within clusters, but a relevant proportion of migrants also occurred between the Moleques and FN/Rocas colonies (Fig. 3).

Environmental variables showed high heterogeneity (Fig. 2). Isotopic niche width ranged from 0.09‰^{2} in boobies from FN to 1.29‰^{2} in Abrolhos (Fig. S4). Significant correlation among environmental variables was observed between Chlα-SST, Chlα-AT, and SST-AT (*P*-value < 0.01; Table S1), and therefore only Chlα was used in subsequent

analyses to avoid multicollinearity. Correspondence between genetic and geographical matrices was positive but not significant (R^2 = 0.202; *P*-value = 0.187), while correspondence between genetic and DisCoast matrices was positive and significant (R^2 = 0.925; *P*-value < 0.01; Table S2). Posterior probability was low in all Bayesian GLMs; the null model had the lowest probability, whereas the GLM fitted with Density+DisCoast had the highest posterior probability (Table S3). The saturated model (DisCoast+Chlα+Density+SEAc) was significant, explaining genetic diversity in the RDA (*P*-value = 0.027), but the reduced model contained only Density+DisCoast (*P*-value = 0.008) (Fig. 4).

Discussion

WITHIN-POPULATION DIVERSITY

In general, brown booby populations exhibited low genetic diversity, with ~3 alleles and an H_O of ~0.4 on average. Although low, allelic richness and heterozygosity were consistent with those of other brown booby colonies (Morris-Pocock, Anderson & Friesen 2011), suggesting that brown booby populations could persist with low genetic diversity. Nevertheless, boobies from SPSP presented remarkably low genetic diversity, suggesting additional evolutionary processes are influencing that population, such as founder effect or a recent bottleneck event.

Mutation-drift equilibrium models were significant, detecting a bottleneck in Rocas, and marginally significant for SPSP, which could be influenced by the high number of monomorphic loci observed in SPSP. Selectively neutral alleles at high frequencies can be fixed (i.e. allele frequency = 1) by random events, such as genetic

drift, mainly in small and isolated populations (Ridley 2003). Therefore, the high homozygosity and percentage of monomorphic loci observed in SPSP could be a result of allelic fixation by genetic drift, or even a recently founded population with already fixed alleles. Nonetheless, the mode-shift distortion observed in SPSP and Rocas suggests a recent population decline, as proposed by Luikart *et al.* (1998).

A likely explanation for population size reduction in SPSP and Rocas could be the exploitation of birds as fresh food by sailors over the past few centuries. In Brazil, hunting seabirds for food was illustrated in the diaries of sailors over the past centuries (d'Abbeville, Métraux & Lafaye 1963; Markham 2011) as, for example, in the Beagle Diary of Charles Darwin when landing at SPSP and Abrolhos (Keynes 2001). Considering 20–30 years as the generation time for brown boobies (Schreiber & Burger 2001) and 10 generations as the interval in which mode-shift distortion could be detected (Luikart *et al.* 1998), a bottleneck would have occurred 200–300 years ago. Therefore, population size reduction by hunting could be a potential explanation for the mutation-drift disequilibrium observed in SPSP and Rocas, which were located on the West Indies trade route. Alternatively, inbreeding could be a relevant factor in maintaining high homozygosity, since SPSP is a small and isolated population. However, F_{IS} calculations were not consistent with this scenario, probably because this coefficient includes only loci with allele frequency $\neq 1$. Long-term mark-recapture studies could address inbreeding at SPSP, a feasible methodology in that archipelago due to the easy access to the nests.

THE ROLE OF IBD ON BROWN BOOBY DIVERSITY

Bayesian estimates suggested that brown boobies in the southwestern Atlantic Ocean are divided into two clusters, while complementary approaches found a weaker, but relevant, subdivision in one of these clusters. Although the IBD model was not able to explain genetic diversity, geographical distance seems to indirectly influence between-colony genetic distance by directly influencing within-cluster environmental similarities. For example, the lowest pairwise geographical distances were found between Rocas and FN, as well as within coastal colonies, which are influenced by similar oceanographic dynamics. Therefore, decreased geographical distances between archipelagos tend to represent similar environmental pressures, reducing selection against immigrants and promoting gene flow from nearby colonies. On the other hand, SPSP is relatively close to FN and Rocas, but genetic distances are among the highest, suggesting the importance of additional drivers of population isolation.

Interestingly, a trend of northward dispersal was observed in the coastal cluster, with high emigration rates from Moleques. Moleques was also shown to contribute 6% and 8% of each generation to Rocas and FN, respectively. Brown boobies from Moleques are known to strongly depend upon shrimp fishery bycatch as food sources (Branco *et al.* 2005), so that annual fluctuations in shrimp catches influence the number of eggs laid by boobies (Branco, Fracasso & Moraes-Ornellas 2013). Foraging plasticity to deal with environmental changes was demonstrated in brown boobies in the Gulf of California, where diving depths and prey size are suggested to be annually adjusted according to environmental fluctuations (Castillo-Guerrero *et al.* 2016). Therefore, considering brown boobies have opportunistic feeding habits and high plasticity, annual fluctuations in food availability could be influencing the northwards dispersal of boobies from Moleques.

Individuals banded in Moleques have already been recaptured at the latitudes of Cagarras, Abrolhos, Rocas and FN (Efe *et al.* 2006), reinforcing the idea that geographical distance is not a barrier for population isolation.

IBE PROMOTING OR DISRUPTING GENE FLOW

The pattern of population differentiation observed in the six colonies of brown boobies showed strong evidence that ecological factors and local adaptation are influencing contemporary gene flow between clusters. The two-cluster arrangement suggested by the Bayesian approach may be explained by the unusual conditions in SPSP, such as high population density and remarkable seascape. Additionally, splitting between FN/Rocas and coastal colonies could be related to oceanographic conditions, and consequently their specialization in using local resources.

Obviously, distance from the mainland by itself has not had a direct influence on the genetic structure of brown boobies, but may represent a set of oceanographic variables. SPSP is a very small and remote archipelago and has oceanographic dynamics similar to those of seamounts. Briefly, SPSP is influenced both by the South Equatorial Current and the Equatorial Undercurrent, which flow in opposite directions generating a mixed layer of highly productive waters in its very near surroundings (Souza *et al.* 2013). FN and Rocas are also outside the Brazilian continental shelf, but are influenced by the oligotrophic South Equatorial Current (Longhurst *et al.* 1995). Moleques, Cagarras, and Abrolhos, in turn, are closest to the mainland, are located on the continental shelf, and their ocean regimes are strongly influenced by the coastal environment. Therefore, the role of DisCoast in explaining genetic diversity could be biased by the large difference between SPSP and the remaining colonies in distance from the coast, which implies tacit

differences in relation to oceanographic dynamics that can lead to increased local adaptation, a mechanism of population isolation in seabirds (Friesen 2015).

In addition to differences in seascape features, species-specific behavior could be contributing to local adaptation and population isolation. Brown boobies usually occupy regions of high productivity around colonies during the breeding period, but present high plasticity in relation to foraging areas as they follow prey availability (Nelson 2005; Castillo-Guerrero *et al.* 2016). Breeding in SPSP is non-seasonal (Barbosa-Filho & Vooren 2010) and foraging trips of breeding adults last about 1 h, which take place within a ~9-km radius around the colony (Nunes *et al.* in prep.). Their diet is based on five flying-fish species (Both & Freitas 2001). Comparatively, brown boobies from Anguilla, an island in the Caribbean, feed within a ~40-km radius around the colony, with a total trip duration of ~5:30 h (Soanes *et al.* 2016), while boobies from Isla San Jorge, Gulf of California, prey on up to 36 species (Castillo-Guerrero *et al.* 2016). These differences in foraging behavior, along with the year-round breeding activities, illustrate that there is plenty of food nearby SPSP throughout the year, suggesting that it is not necessary for the boobies to fly far away from the colony, even during the non-breeding period, to obtain food. Although there is no information about the non-breeding distribution of brown boobies from SPSP, the high number of non-breeding individuals using SPSP as a resting site suggests that boobies stay in the colony throughout the year (Barbosa-Filho & Vooren 2010), which is also suggested to be a factor for gene flow disruption in seabirds (Friesen 2015).

Additionally, landscape heterogeneity and availability of nesting areas directly influence nest density, which in turn seems to be affecting the genetic diversity of brown boobies in the southwestern Atlantic Ocean. Brown boobies prefer flat and slightly

sloping ground on the edge of cliffs for nest building, and colonies are composed of scattered groups of nests with irregular spacing, which usually ranges from 0.6 to 27 m (Nelson 2005). This is the case of brown boobies from Moleques (Branco, Fracasso & Moraes-Ornellas 2013) and Cagarras (Alves *et al.* 2004), which prefer slightly sloping areas, while in Abrolhos, boobies preferably nest on cliff edges (Alves *et al.* 2000). Conversely, brown boobies from SPSP are mainly based in Belmonte Island, which comprises 6000 m^2, a maximum altitude of 21 m, and ~250 nests located on the highest portion of the island in an area of only ~700 m^2 (Barbosa-Filho & Vooren 2010). This aggregation results in a high-density colony with an average between-nest distance of ~1 m (Kohlrausch 2003), generating fierce competition for space that causes injuries to adults and chicks, including cannibalism (Neves *et al.* 2015). Therefore, living in SPSP apparently requires an ability to compete for nesting areas, which could promote population isolation by local adaptation and selection against immigrants.

Although genetic differences between FN/Rocas and coastal colonies were weaker, a fine-scale seascape could influence the reduced gene flow observed between such clusters. In general, tropical ocean waters tend to have low primary productivity due to the stability in temperature stratification (Moreno-Ostos *et al.* 2011). In these regions, productivity peaks are mainly associated with topography, creating a patchy pattern of nutrient distribution, as observed along the southwestern Atlantic Ocean (Souza *et al.* 2013). Conversely, neritic environments are characterized by increased primary productivity, as they are influenced mainly by the input of nutrients from river outflows (Santos *et al.* 2008), continental shelf fronts (Acha *et al.* 2004), and vortices generated by winds and slope topography (Odebrecht & Castello 2001). Moleques, Cagarras and Abrolhos are influenced by such processes. Around Moleques, however, waters are

fertilized by the Rio de la Plata plume (Möller-Jr *et al.* 2008) and are influenced by the Subtropical Shelf Front (Piola, Romero & Zajaczkovski 2008), which are key processes for the high local productivity (Odebrecht & Castello 2001). Accordingly, input of nutrients around Cagarras is driven mainly by Guanabara Bay runoff, which is highly eutrophic (Kjerfve, Lacerda & Dias 2001), and the Cabo Frio upwelling system, which is an ascending process of the South Atlantic Central Water (Valentin 2001). Finally, boobies from Abrolhos depend on the region influenced by the Caravelas River (Pereira 2012), a nutrient-rich area supporting the second most complex mangrove system in the northeastern region of Brazil (Herz 1991).

Despite their sophisticated apparatus for diving, brown boobies are widely known for their foraging plasticity, including taking advantage of fisheries discards (Carboneras 1992). In the coastal colonies studied here, fisheries discards are common and the diet of brown boobies is composed of a wide range of species, such as benthic species, which are unreachable by diving (Alves *et al.* 2004; Branco *et al.* 2005). Contrastingly, the diet of SPSP boobies is usually composed of five flying-fish species (Both & Freitas 2001), since there is no regular commercial fishing in the foraging area of these boobies around SPSP, and consequently, there is no dependence upon fisheries discards. Therefore, between-colony diet differences are primarily associated to what is available around colonies, as it has been demonstrated by regurgitate analysis.

Nonetheless, isotopic data were not able to explain the population structure of brown boobies in the southwestern Atlantic Ocean, what could be associated to shortcomings of the technique and sampling design. Firstly, the comparison of isotopic niches is sensitive to differences in isotopic composition of resources between distinct systems, such as neritic vs. pelagic (Newsome *et al*. 2007). A potential solution would be

transforming δ data in relative proportions of distinct resources incorporated into boobies blood (p-space; Newsome *et al*. 2007), but isotopic data from potential prey for each colony are lacking. Second, substantial difference was observed between Abrolhos and the remaining colonies, including the coastal ones, which were expected to have similar niche width. This could be associated to the compreehensive sampling carried out during both summer and winter seasons in Abrolhos (Mancini, Hobson & Bugoni 2014), while Cagarras and Moleques were sampled only during summer. Seasonal variations in environmental conditions are more pronounced in the continental shelf than in the pelagic tropical archipelagos, where oceanographic dynamics tend to be stable throughout the year (Soares *et al*. 2012; Souza *et al*. 2013). From this, isotopic values are known to vary in space and time in coastal colonies associated with oceanographic processes, requiring a sampling design to comply with such shortcomings (Kurle & McWhorter, 2017). Therefore, sampling brown boobies at Moleques and Cagarras during both summer and winter could make isotopic niche width of coastal colonies more comparable and similar, contributing to explain genotypic differences between FN/Rocas and coastal colonies.

In summary, ecotype-based clustering among populations of a widespread seabird has been demonstrated, suggesting that IBE is a stronger driver of population differentiation than IBD, and therefore that seabirds can be useful model organisms to predict responses of wild populations to climate change. The increased plasticity of foraging strategies, along with tolerance to a wide range of environmental conditions make brown boobies potential "winners" under climate change scenarios, as already suggested for other seabird species (e.g. LaRue *et al*. 2013; Precheur *et al*. 2016). However, colonies could be differentially impacted according to their local environmental characteristics, such that low primary productivity due to SST anomalies

could negatively impact colonies already surrounded by oligotrophic waters, such as FN, Rocas and SPSP, by decreasing prey availability (Grémillet & Boulinier 2009). Additionally, impacts on SPSP boobies could be strong, since it is a genetically and geographically isolated population with low genetic diversity, depends on a small-scale oceanographic system with no alternative foraging zone, and since the colony has a small nesting area that ranges from only 10.7 m to 20.7 m a.s.l., exposing it to sea level rise. This study demonstrates that identifying evolutionary processes of population differentiation should be of paramount importance for management programs, since knowing within- and between-colony relationships is crucial for defining local and global conservation priorities.

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Table 1. Genetic diversity based on nine microsatellite loci for six colonies of brown boobies (*Sula leucogaster*) distributed in the southwest Atlantic Ocean. H_O = observed heterozygosity; H_E = expected heterozygosity from Hardy-Weinberg proportions (Nei 1978); F_{IS} = inbreeding coefficient (Weir and Cockerham 1984); and A = allelic richness. SPSP = Saint Peter and Saint Paul; FN = Fernando de Noronha; Rocas = Rocas Atoll; Moleques = Moleques do Sul. Bold values were deviated from Hardy-Weinberg equilibrium.

Loci	SPSP (♀=12; ♂=12)				FN (♀=8; ♂=11)				Rocas (♀=12; ♂=8)				Abrolhos (♀=11; ♂=9)				Cagarras (♀=10; ♂=9)				Moleques (♀=9; ♂=9)			
	H_O	H_E	F_{IS}	A	H_O	H_E	F_{IS}	A	H_O	H_E	F_{IS}	A	H_O	H_E	F_{IS}	A	H_O	H_E	F_{IS}	A	H_O	H_E	F_{IS}	A
Sv2A-2	-	-	-	1	0.33	0.36	0.06	2	0.44	0.51	0.14	2	0.30	0.26	-0.15	2	**0.74**	0.50	-0.47	2	0.50	0.51	0.02	2
Sv2A-26	0.46	0.47	0.02	2	0.44	0.46	0.04	4	0.47	0.44	-0.08	4	0.25	0.34	0.28	4	0.16	0.15	-0.04	3	0.33	0.29	-0.17	2
Sn2A-123	0.42	0.42	0.01	2	0.39	0.32	-0.21	2	0.41	0.45	0.09	2	0.40	0.49	0.19	2	0.39	0.39	-0.01	2	0.39	0.51	0.24	2
Sn2B-83	-	-	-	1	0.39	0.32	-0.21	2	0.58	0.46	-0.25	2	-	-	-	1	-	-	-	1	0.06	0.06	0.00	2
Sv2A-95	-	-	-	1	0.16	0.15	-0.06	2	-	-	-	1	0.37	0.42	0.13	2	0.17	0.25	0.32	2	0.29	0.26	-0.14	2
Sv2A-47	-	-	-	1	0.63	0.69	0.09	4	0.71	0.66	-0.06	4	0.70	0.61	-0.14	3	0.63	0.61	-0.02	3	0.47	0.48	0.03	3
Sn2B-100	0.04	0.04	0.00	2	0.22	0.29	0.22	2	0.29	0.40	0.27	2	0.40	0.38	-0.04	2	0.33	0.36	0.06	2	0.28	0.25	-0.13	2
Sv2B-138	-	-	-	1	0.73	0.71	-0.03	6	**0.69**	0.82	0.15	7	0.90	0.72	-0.25	5	0.71	0.66	-0.08	6	0.62	0.64	0.04	6
Sv2B-27	0.65	0.51	-0.29	2	0.43	0.42	-0.01	2	0.76	0.51	-0.51	2	0.40	0.34	-0.16	3	0.24	0.30	0.21	2	0.18	0.17	-0.06	2
Mean	0.17	0.16	-0.09	1.4	0.41	0.41	0.00	2.9	0.49	0.47	-0.03	2.9	0.41	0.40	-0.04	2.7	0.37	0.36	-0.04	2.6	0.35	0.35	0.02	2.6

Table 2. Genetic and geographical distances between six colonies of brown boobies (*Sula leucogaster*) in the southwest Atlantic Ocean. Pairwise Nei's F_{ST} are values below the diagonal, and pairwise geographical distance (km) are above the diagonal. Bold values represent significant genotypic differentiation based on Fisher's exact test, with adjusted *P*-values based on the Bonferroni correction for multiple comparisons (*P*-values < 0.001). SPSP = Saint Peter and Saint Paul Archipelago; FN = Fernando de Noronha Archipelago; Rocas = Rocas Atoll; Moleques = Moleques do Sul.

SPSP	570	671	2021	2626	3242
0.272	FN	153	1452	2058	2684
0.250	0.036	Rocas	1375	1960	2571
0.295	**0.054**	**0.072**	Abrolhos	655	1348
0.358	**0.057**	**0.054**	**0.049**	Cagarras	700
0.339	**0.085**	**0.072**	**0.044**	0.022	Moleques

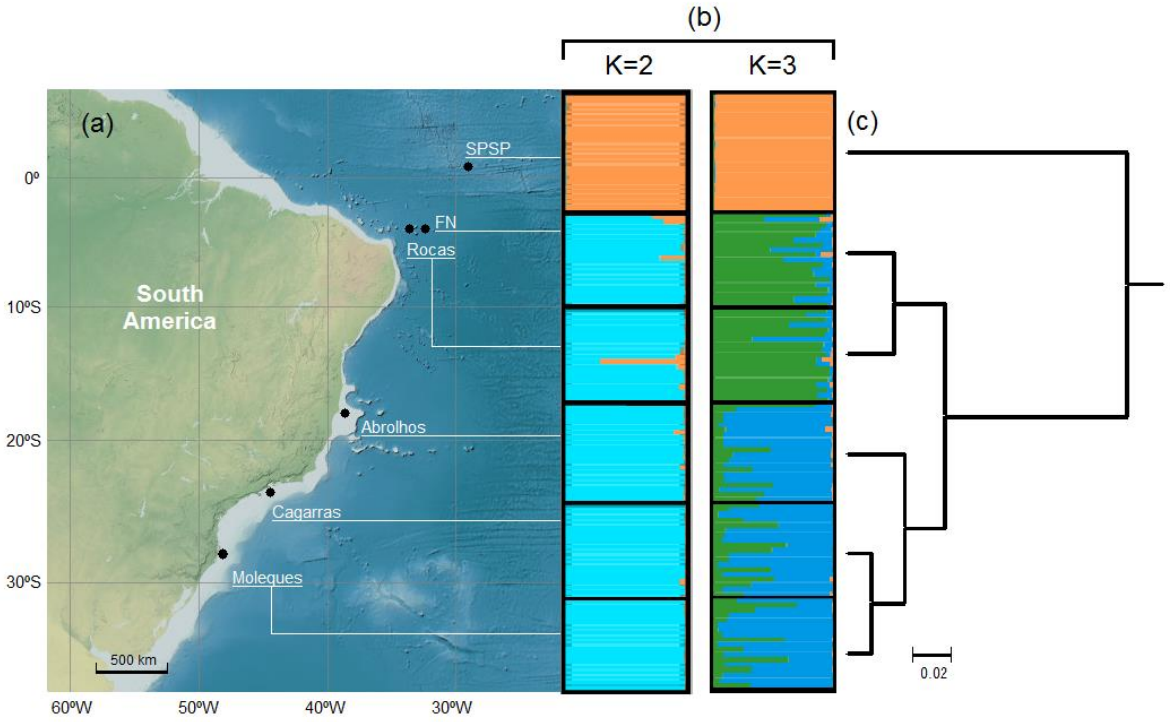

Fig. 1. Study area with cluster arrangements based on genetic data. **A.** Brown booby, *Sula leucogaster*, colonies in the southwestern Atlantic Ocean **B.** Bar plots from Bayesian estimates of population structure based on microsatellite data considering two (K = 2; left side) and three clusters (K = 3; right side). **C.** Phylogenetic tree built through the UPGMA method from pairwise genetic distances (Nei's F_{ST}). SPSP = Saint Peter and Saint Paul Archipelago; FN = Fernando de Noronha Archipelago; Rocas = Rocas Atoll; Moleques = Moleques do Sul. For references to colour, see the online version.

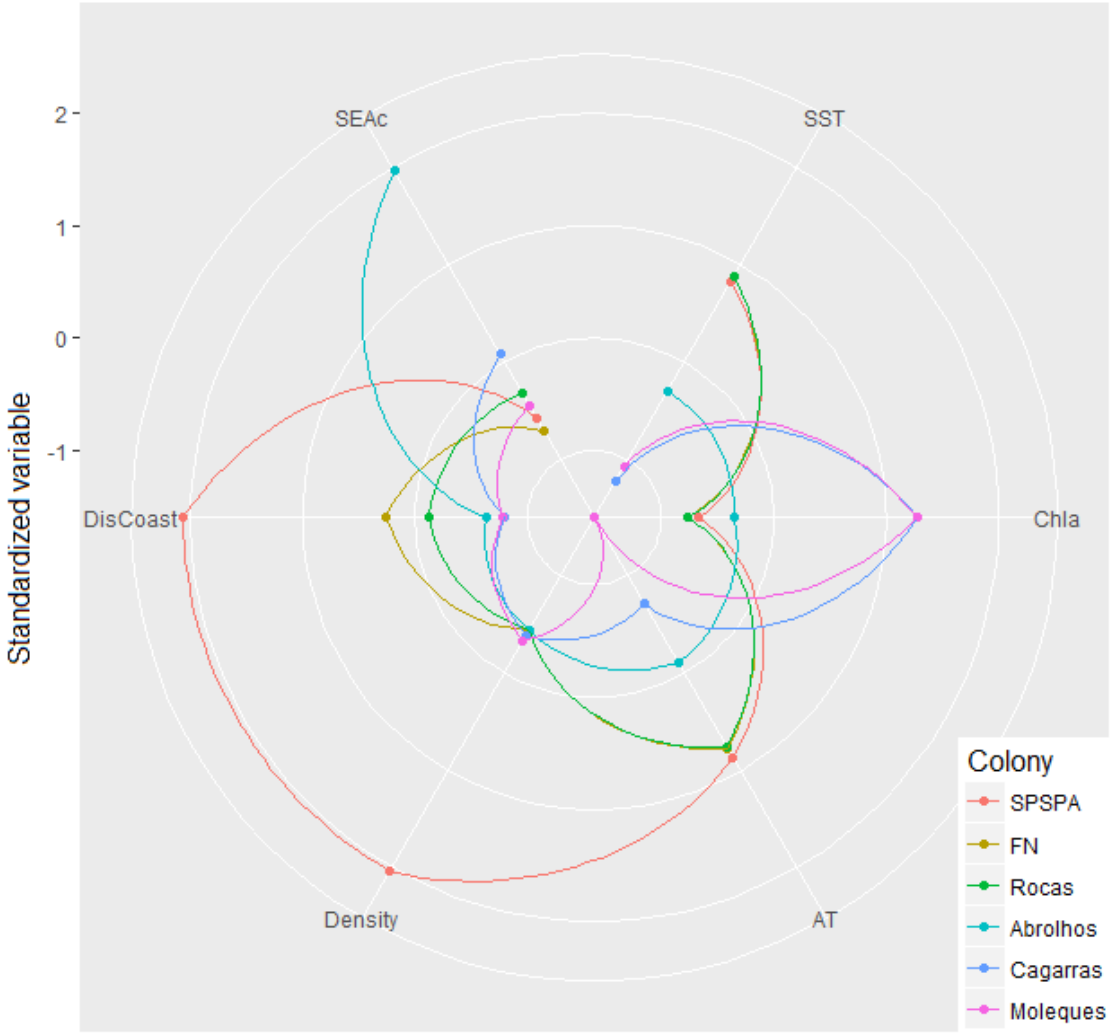

Fig. 2. Radial plot demonstrating heterogeneity of seascape features and colony-specific parameters across the six brown booby colonies in the southwestern Atlantic Ocean. Each radial line corresponds to a standardized environmental variable with values decreasing towards the centre. Each point on radial lines corresponds to the population mean (the ellipse '0' corresponds to the mean for each variable). Chlα = chlorophyll α; SST = sea surface temperature; AT = air temperature; DisCoast = minimum distance from the mainland; Density = population density; SEAc = standard Bayesian ellipse areas from isotopic data. For references to colour, see the online version.

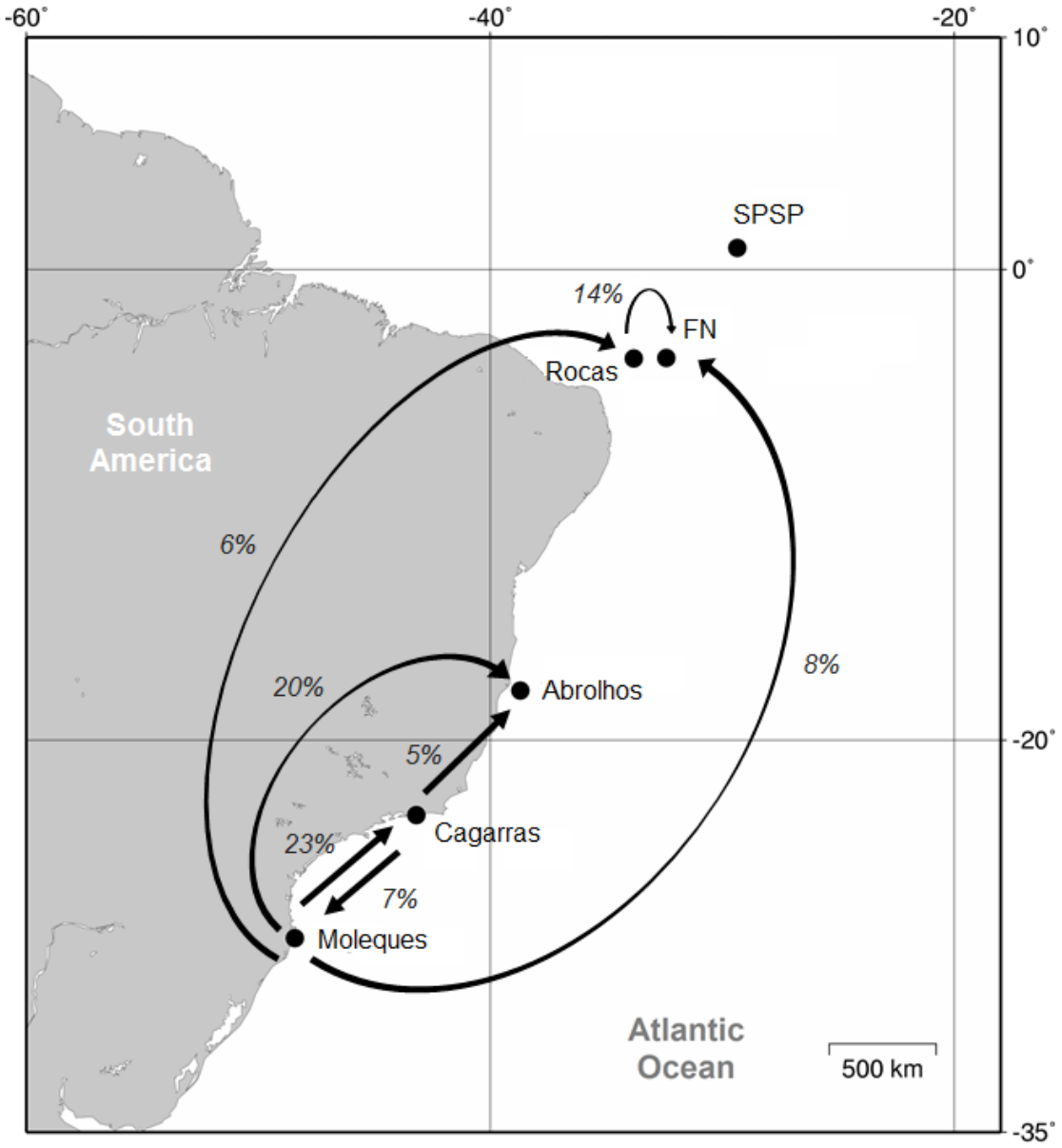

Fig. 3. Bayesian inference of dispersal rates among the six brown booby colonies in the southwestern Atlantic Ocean. Gene flow is represented by the fraction (%) of individuals in a given population that are immigrants from the remaining populations. SPSP = Saint Peter and Saint Paul; FN = Fernando de Noronha; Rocas = Rocas Atoll; Moleques = Moleques do Sul.

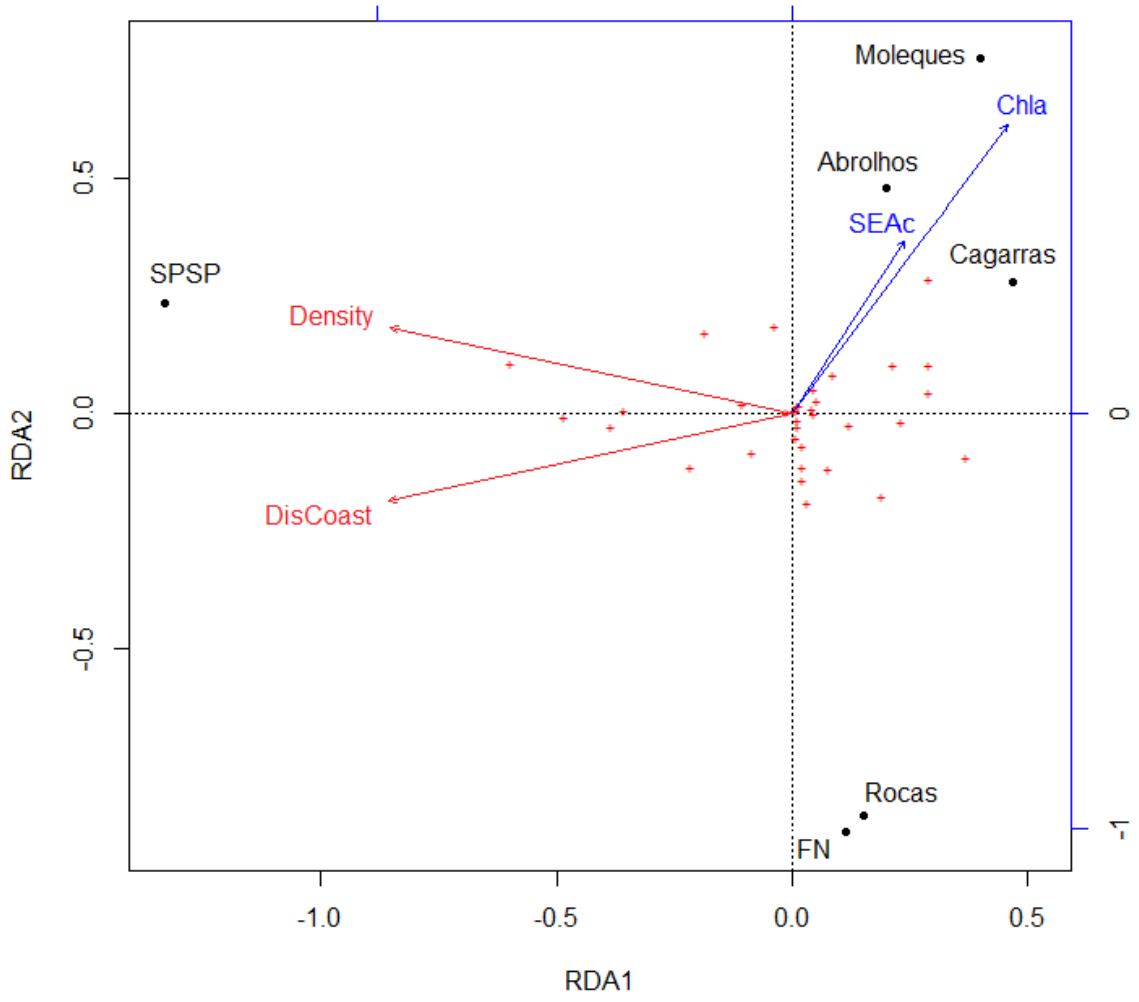

Fig. 4. Redundancy analysis demonstrating how environmental variables correspond to genetic variation of the six brown booby colonies. Angles between arrows are defined by Pearson's correlation and direction of a projected arrow indicates where are the highest values. Red variables and arrows (Density and DisCoast) represent which environmental variables better explain variations in allele frequencies among colonies. SPSP = Saint Peter and Saint Paul; FN = Fernando de Noronha; Rocas = Rocas Atoll; Moleques = Moleques do Sul. Chlα = chlorophyll α; SST = sea surface temperature; AT = air temperature; DisCoast = minimum distance from the mainland; Density = population density; SEAc = standard Bayesian ellipse areas from isotopic data.

Supplementary material

Appendix S1. Additional methodological details.

1. DNA extraction and microsatellite amplifications

DNA extraction followed the 5M sodium chloride protocol (Medrano, Aasen & Sharrow 1990). All samples were amplified at nine microsatellite loci with primers previously described by Taylor *et al*. (2010). M13(-21) tail was incorporated into each forward primer at the 5' end (Schuelke 2000) and consequently to the PCR products. PCRs were performed in a 20-µl reaction containing 20–30 ng of DNA, 10 pmol of forward primer, 10 pmol of reverse primer, 10 pmol of fluorescent dye label (HEX or FAM), 10 mM of each dNTP, 1.5 mM of MgCl2, 1x PCR buffer, and 1 unit of *Taq* DNA-polymerase. The following PCR profile was used: 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, annealing temperature for 30 s and 72°C for 30 s, with a final extension in 72°C for 10 min. Annealing temperature was adjusted in: 50°C for Sv2A-95, Sv2A-47 and Sv2B-27; 52°C for Sv2A-2 and Sn2A-123; 54°C for Sn2B-100; and 56ºC for Sv2A-26 and Sn2B-83. For Sv2B-138 we used the touchdown program proposed by Taylor *et al*. (2010). To avoid biased readings between genotyping rounds, ~5–10% of already genotyped samples were reassayed in the subsequent genotyping. PCR products were run on ABI 3730XLs (Applied Biosystems; internal standard size marker 400 HD).

2. Population density calculations

Total area (km^2) for each archipelago was obtained with the ruler tool in Google Earth Pro, by considering the maximum perimeter of emerged portions. Census-based population sizes for SPSP, FN, Rocas, and Abrolhos were obtained from Mancini,

Serafini & Bugoni (2016), and data for Cagarras and Moleques were obtained from Cunha *et al*. (2013) and Branco, Fracasso & Moraes-Ornellas (2013), respectively.

3. Carbon and nitrogen isotopic data used for calculations of isotopic niche width

For calculating isotopic niche width, a previously published data set of carbon and nitrogen isotopic ratios for SPSP, FN, Rocas, and Abrolhos was used, which is based on blood samples obtained from breeding adults in 2010 and 2011 (Mancini, Hobson & Bugoni 2014). Additionally, samples of whole blood were taken from boobies breeding in Cagarras and Moleques in 2014 and processed in the laboratory following procedures described in Mancini, Hobson & Bugoni (2014). Because data from Mancini, Hobson & Bugoni (2014) were analyzed at a distinct laboratory, laboratory-biased results were assessed by running Pearson's correlation and *t*-test with 10 samples of yellow-nosed albatross *Thalassarche chlororhynchos* (Gmelin, 1789), which were sent to both laboratories. Samples from the inter-laboratory comparison demonstrated a high correlation for carbon (R^2 = 0.86; *P*-value < 0.001) and nitrogen (R^2 = 0.97; *P*-value < 0.001). Similarly, deviations were not detected between means through a paired t-test for carbon (t = -1.29; df = 17.99; *P*-value = 0.21) and nitrogen (t = 0.19; df = 17.99; *P*-value = 0.84). Thus, samples from different labs were pooled for subsequent analysis. Stable isotope ratios were expressed in δ notation as parts per thousand (‰) differences from the international reference material Vienna Pee Dee Belemnite (VPDB) limestone for carbon and air for nitrogen.

4. References

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Table S1 Pairwise Pearson's correlations (R^2) among environmental variables of six brown booby colonies along the southwestern Atlantic Ocean. Chlα = chlorophyll α concentration; SST = sea surface temperature; AT = air temperature; DisCoast = minimum distance from the mainland; Density = population density; SEAc = Bayesian ellipse areas estimated from isotopic data of carbon and nitrogen. Bold values represent correlations with *P*-value < 0.01.

Chlα					
0.903	SST				
0.865	**0.884**	AT			
0.360	0.490	0.476	DisCoast		
0.084	0.137	0.152	0.828	Density	
0.010	-0.321	-0.178	-0.426	-0.291	SEAc

Table S2. Pairwise Pearson's correlations (R^2) between distance matrices calculated with Mantel tests. GenDiv = genetic diversity; GeoDis = geographical distances between archipelagos; Chlα = chlorophyll α concentration; SST = sea surface temperature; AT = air temperature; DisCoast = minimum distance from the mainland; Density = population density; SEAc = Bayesian ellipses area based on carbon and nitrogen isotopic ratios. GenDiv matrix was based on linearized F_{ST} (Slatkin 1995), GeoDis was calculated considering log-transformed geographical distances, and distance matrices for the remaining variables were calculated with the Mahalanobis dissimilarity index. Bold values represent *P*-values < 0.01.

GenDiv							
0.202	GeoDis						
0.000	**0.511**	Chlα					
0.005	**0.785**	**0.741**	SST				
0.003	**0.748**	0.667	0.699	AT			
0.925	0.099	0.002	0.046	0.020	DisCoast		
0.941	0.002	0.032	0.014	0.013	0.841	Density	
-0.207	0.092	-0.198	-0.068	-0.184	-0.192	-0.214	SEAc

Tabela S3. Outputs of Bayesian generalized linear model fitting, using all the possible combinations of environmental variables to explain population-specific F_{ST}'s. Model highlighted in bold had the highest posterior probability. Chlα = chlorophyll α; DisCoast = minimum distance from the mainland; Density = population density; SEAc = standard Bayesian ellipse areas from isotopic data.

Explanatory variables (factors)	Posterior probability
Constant (null model)	0.0561
Constant, Chlα	0.0578
Constant, DisCoast	0.0676
Constant, DisCoast, Chlα	0.0627
Constant, Density	0.0595
Constant, Density, Chlα	0.0614
Constant, Density, DisCoast	**0.0696**
Constant, Density, DisCoast, Chlα	0.0649
Constant, SEAc	0.0622
Constant, SEAc, Chlα	0.0572
Constant, SEAc, DisCoast	0.0622
Constant, SEAc, DisCoast, Chlα	0.0649
Constant, SEAc, Density	0.0626
Constant, SEAc, Density, Chlα	0.0600
Constant, SEAc, Density, DisCoast	0.0672
Constant, SEAc, Density, DisCoast, Chlα	0.0640

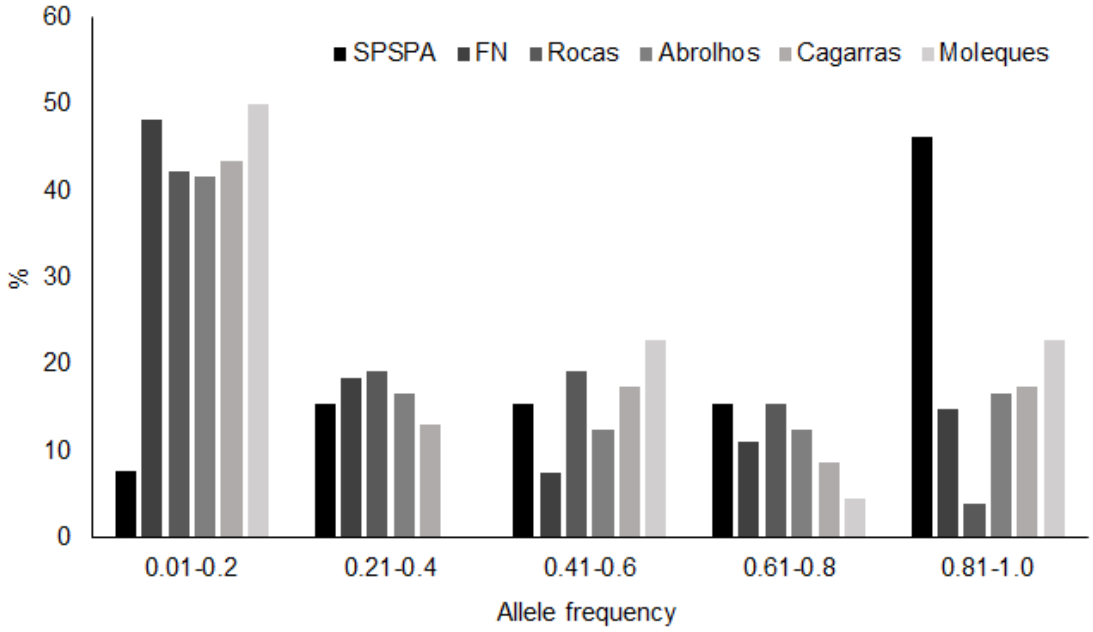

Fig. S1. Proportion of each allele frequency range for the brown booby (*Sula leucogaster*) colonies in the southwestern Atlantic Ocean. L-shaped distributions indicate populations bottlenecked less than about a dozen generations ago. SPSP = Saint Peter and Saint Paul Archipelago; FN = Fernando de Noronha Archipelago; Rocas = Rocas Atoll; Moleques = Moleques do Sul Archipelago.

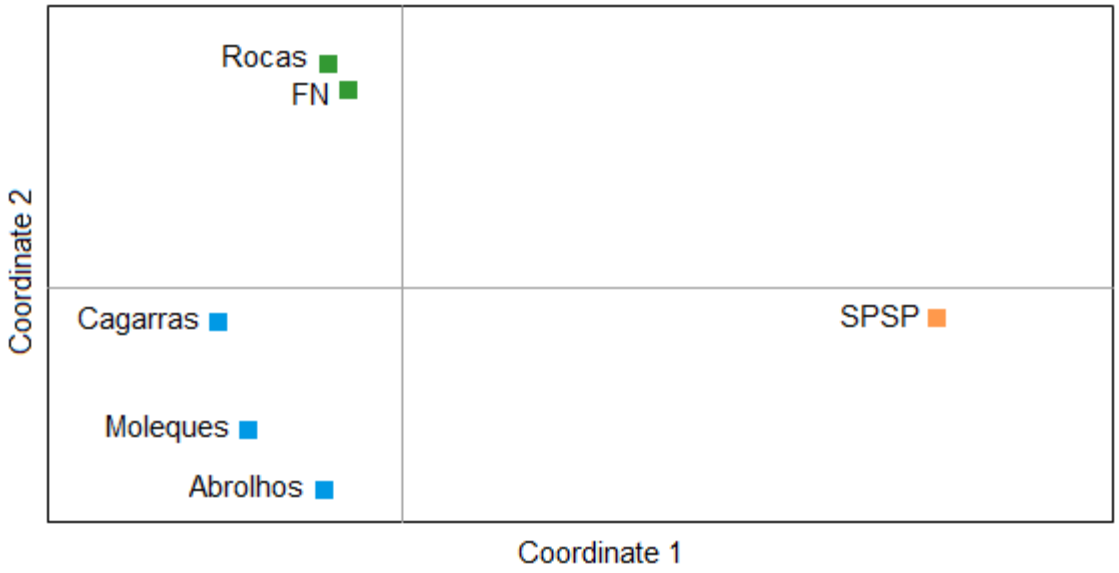

Fig. S2. Multivariate bidimensional clustering based on pairwise genetic distances (Nei's F_{ST}) provided by Principal Coordinate Analysis. The two principal coordinates explained 93% of the total variance. SPSP = Saint Peter and Saint Paul Archipelago; FN = Fernando de Noronha Archipelago; Rocas = Rocas Atoll; Moleques = Moleques do Sul Archipelago.

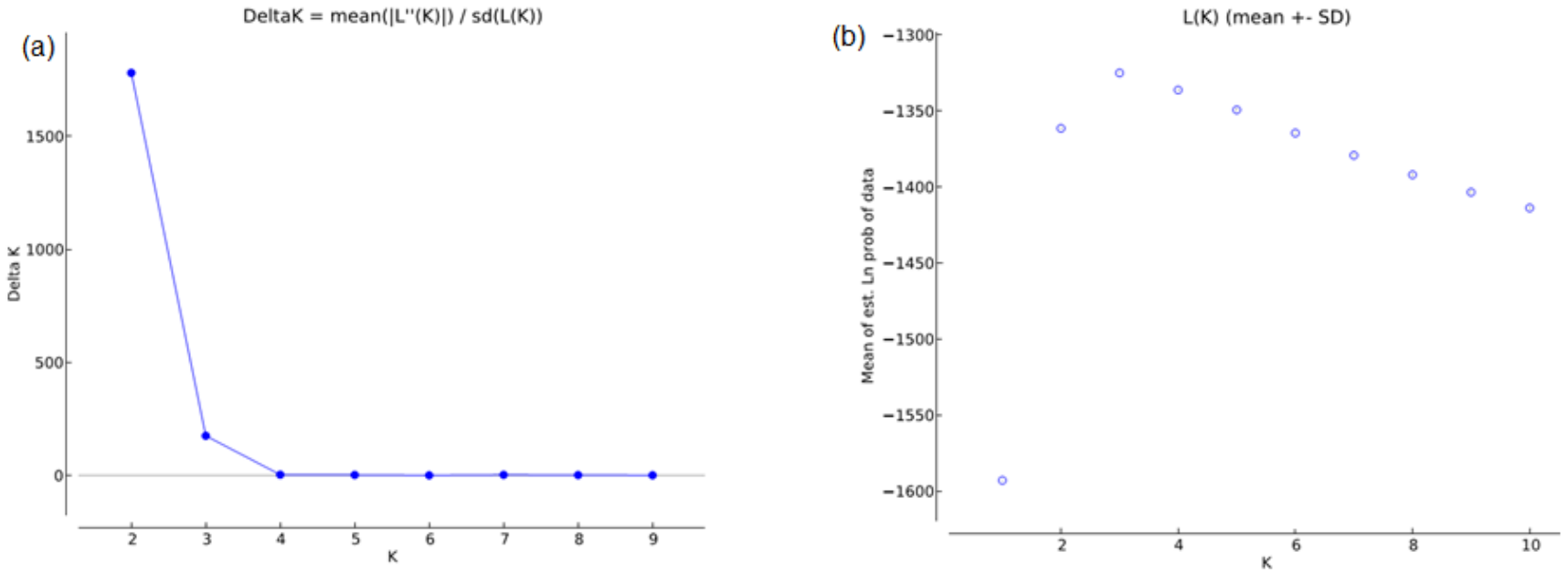

Fig. S3. (a) Method used for detecting the best number of clusters based on Evanno, Regnaut & Goudet (2005); (b) Mean likelihood L(K) and variance per K value from STRUCTURE data.

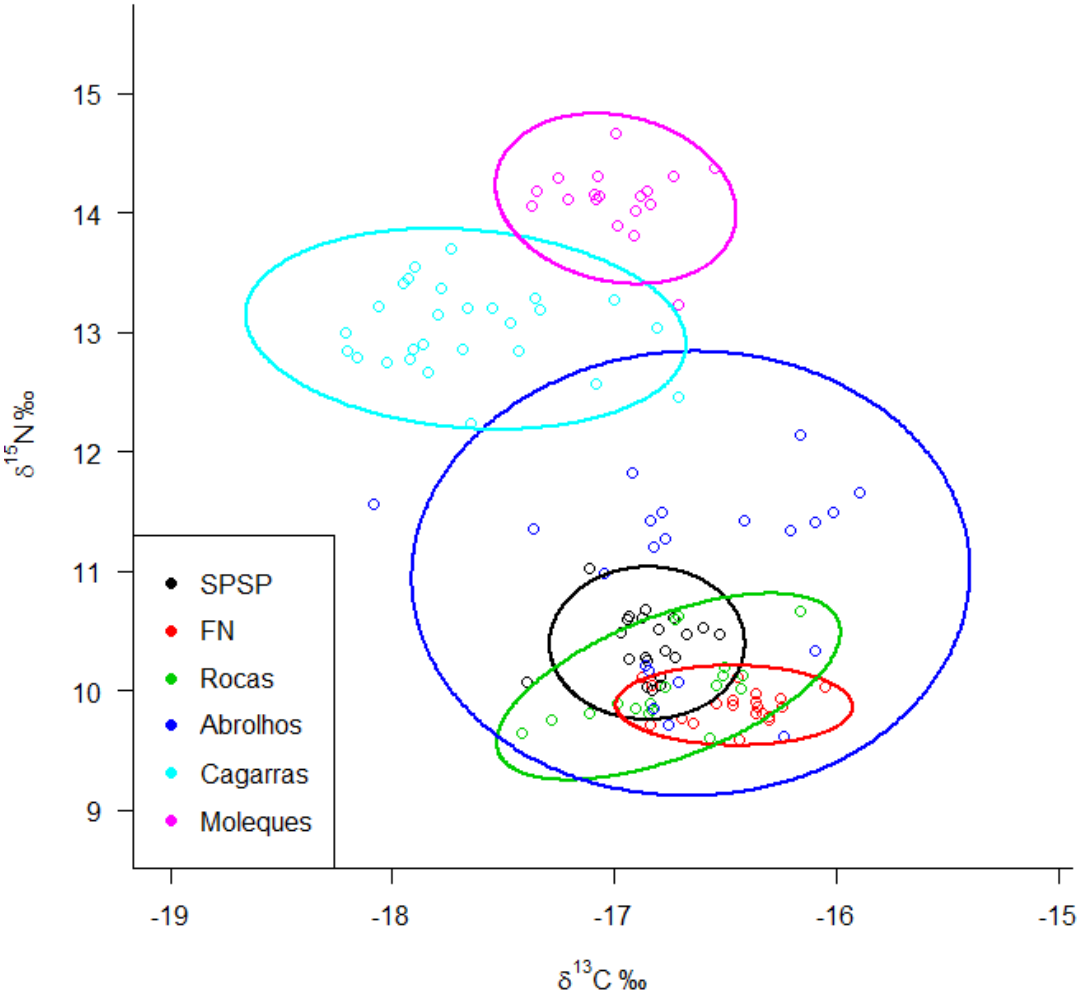

Fig. S4. Bayesian ellipses based on isotopic data of carbon and nitrogen with 95% confidence interval and corrected for small sample size, as implemented in the package SIBER. SPSP = Saint Peter and Saint Paul Archipelago; FN = Fernando de Noronha Archipelago; Rocas = Rocas Atoll; Moleques = Moleques do Sul Archipelago.

Anexo 3

Seabirds fighting for land: fitness consequences of breeding area constraints at a small remote archipelago

Formatado de acordo com as normas do periódico *Oecologia*

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Declaration of authorship: GTN and LB originally formulated the idea and developed methodology, GTN conducted fieldwork, GTN and SB performed statistical analyses, and GTN wrote the manuscript.

Abstract The identification of associations between phenotypes and environmental parameters is crucial to understand how natural selection acts at individual level. Inter-population cohesive forces (e.g. gene flow) may hinder effects of local selective pressures, yet genetically isolated populations can be useful models for identifying the forces selecting fitness-related traits. In the present study, we carried out a comprehensive sampling on a genetically and ecologically isolated population of the strictly marine bird, the brown booby *Sula leucogaster*, at the tropical remote Saint Peter and Saint Paul Archipelago. The aim was to detect phenotypic adjustments from inter-individual differences in diet, foraging behavior, and nest quality. We qualified all the 112 active nests in 2014 and 2015 by their landscape characteristics, and took morphometrics and body mass measurements from 319 individuals. In addition, we sampled 97 individuals for diet parameters, through samples of regurgitate material and stable isotopes of carbon and nitrogen. We also tracked the same individuals with GPS dataloggers, in order to obtain information on their foraging behavior. While body size was not related to foraging parameters, body size of females (responsible for nest acquisition and defense) was significantly associated to nest quality, as larger females occupied high-quality nests. The remote archipelago has only ~0.017 km^2 and is influenced by seamount-like seascape, concentrating food resources in their very near surroundings. Therefore, we suggest that the small breeding area is a limiting factor rather than prey availability, shedding light on the role of on land features in shaping phenotypic characteristics and fitness in land-dependent marine vertebrates.

Keywords Brown booby, Intra-population diversity, Stable isotopes, Remote tracking, Nest quality.

Introduction

Identifying associations between phenotypic variability and local selective pressures at population level is crucial to understand population differentiation, intraspecific diversity, and to predict sensitivity to environmental changes (Siepielski et al. 2009; Grant and Grant 2014). Nonetheless, gene flow and genetic drift, for example, may hinder the role of local pressures at population level, making necessary the identification of patterns of population structure and stochastic processes before tracking population-specific drivers of local adaptation (Kawecki and Ebert 2004; Bolnick and Nosil 2007). In other words, for closely related populations with contemporary genotypes shared by gene flow, phenotypic diversity is adjusted as a balance between adaptation to local conditions and heritage from successful immigrants; but fitness-related traits of genetically isolated populations are, in turn, strongly dependent on local selective pressures (Mayr 1956). Therefore, isolated populations are influenced by local conditions rather than inter-population cohesive forces (e.g. gene flow) and can even deviate from ecogeographical rules (e.g. Bergmann's rule, Nunes et al. 2017), arising as useful models to explore the role of environmental features as selective forces for phenotype adjustment in wild populations (Reznick and Ghalambor 2005).

Effects of spatiotemporal environmental differences on phenotypic diversity have been extensively demonstrated in natural populations, which have been used as open air laboratories for understanding microevolutionary processes at the individual level (Hendry and Kinnison 2001; Reznick and Ghalambor 2005). Seminal research projects demonstrated fine associations between morphological traits and environmental correlates: the role of predation pressure shaping color patterns of male guppies in South American streams (Reznick and Endler 1982), rainfall and seed availability shifting bill size frequencies in Darwin's finches from Galapagos (Price et al. 1984; Grant and Grant 2002) and perch height and diameter selecting number of subdigital lamellae and leg size of *Anolis* lizards in Caribbean islands (Losos and Sinervo 1989;

Glossip and Losos 1997). Currently, a handful of research tools has been applied to identify individual fitness-related traits and its environmental correlates in evolving natural populations, from fungal prevalence and infection intensity (Lambertini et al. 2016) to adapting molar shape of mammals in islands (Ledevin et al. 2016). Nonetheless, information on the drivers of local adaptation in marine vertebrates is still scarce.

Field studies addressing evolution issues include important trade-offs which are often absent in laboratory studies due to the natural environment complexity (Reznick and Ghalambor 2005), and combining traditional and innovative techniques makes it possible to investigate natural selection even in free-living animals, such as seabirds. Despite their high mobility, seabirds are known to hold phylogeographic structure associated to colony-specific environmental conditions (Friesen et al. 2007; Friesen 2015), presenting gene flow disruption even between sympatric populations (e.g. Hailer et al. 2011). Demographic parameters at population level were demonstrated to be affected by individual breeding quality in seabirds (Lescroël et al. 2009) and, with the advent of innovative remote tracking technologies, individual specialization in at-sea foraging behavior has been shown during the breeding (Wakefield et al. 2015) and non-breeding (Fayet et al. 2016) seasons. In this context, seabirds arise as interesting models for testing individual quality and detecting fitness-related traits and the respective environmental correlates, due to the high intraspecific diversity, high philopatry, colonial breeding, and dependence on resources availability around colonies (Schreiber and Burger 2001).

The brown booby *Sula leucogaster* is a strictly marine bird distributed worldwide, which holds phylogeographic structure and population differentiation even within an ocean basin (Morris-Pocock et al. 2011). Brown boobies nest in oceanic islands directly in small depressions of the ground and, as central-place foragers, depend on prey availability in the surrounding colony for obtaining food (Carboneras 1992). In the southwest Atlantic Ocean, three clusters

of brown boobies were identified and population structure was suggested to be associated to local adaptation due to seascape differences and colony-specific demographic features (Nunes and Bugoni, in review). In addition to being genetically distinct, brown boobies breeding in the remote tropical Saint Peter and Saint Paul Archipelago (hereafter "SPSP"), which is an emerged portion of the Mid-Atlantic ridge, were demonstrated to be the largest and heaviest in comparison to the remaining colonies in the southwest Atlantic Ocean, shedding light on local selective pressures promoting population differentiation (Nunes et al. 2017).

Due to its geographical location, SPSP is influenced by peculiar oceanographic conditions which are similar to seamounts and, thus, the spatially constrained prey distribution can also make distribution of predators limited. This small-scale oceanographic dynamics increases local productivity and concentrates biomass around the archipelago, supplying a rich top predator community of resident species, such as bottlenose dolphins *Tursiops truncatus* (Milmann et al. in press), brown boobies, black noddies *Anous minutus* and brown noddies *A. stolidus* (Mancini and Bugoni 2014), and migratory species, such as whale sharks *Rhincodon typus* (Macena and Hazin 2016), blackfin tunas *Thunnus atlanticus* (Bezerra et al. 2011) and oilfish *Ruvettus pretiosus* (Viana et al. 2012). Additionally, the small emerged area makes the configuration of the brown boobies colony distinct from the pattern observed for this species. Colonies of brown boobies are usually small and composed by scattered groups with irregular spacing (Nelson 2005), but in SPSP nest density is particularly high due to the limited available area for nesting. The ~500 individuals breed in less than 0.006 km^2 (Barbosa-Filho and Vooren 2010; Mancini et al. 2016) and the resulting between-nest distance is only about 1 m (Kohlrausch 2003).

In the present study we used the genetically and ecologically isolated, and phenotypically distinct, brown booby colony at SPSP to investigate the environmental factors that are selecting phenotypes. For this, we used an observational approach not considering a

temporal data series, but extensively documenting phenotypic variation along with nest characteristics, diet and foraging behavior parameters. It is important to highlight that our aim was primarily to identify current associations of individual phenotypes and potential selective pressures rather than describing population-specific microevolutionary processes, such as strength and direction of selection, which requires sampling over a longer time frame (Siepielski et al. 2009). Previous studies demonstrated that genetic and phenotypic distance observed between brown boobies from SPSP and adjacent colonies (i.e. Fernando de Noronha Archipelago and Rocas Atoll) could be potentially explained by local adaptation due to colony-specific selective pressures, highlighting the role of the environmental features in isolating these highly mobile top-predators (Nunes et al. 2017; Nunes and Bugoni in review). Due to the comparatively large mean body size observed in brown boobies from SPSP (Nunes et al. 2017), we hypothesized that differences in foraging parameters (i.e. diet and behavior) and nest characteristics should be observed between small and large individuals, i.e. larger body size should be advantageous in some aspect in SPSP. In other words, in the current study we searched for environmental characteristics shaping behavior, ecology and body size of brown boobies.

Material and methods

Study area

Saint Peter and Saint Paul is a small remote tropical archipelago, composed by ten islets with a total area of 0.017 km^2 and located at 0°55'N and 29°20'W, on the mid-Atlantic ridge. The archipelago holds colonies of brown noddies *Anous stolidus*, black noddies *A. minutus*, and brown boobies, so that the latter breeds mainly on Belmonte Island, which has 21 m of altitude and 6000 m^2 of area (Mancini et al. 2016). Despite the spatial constraint, the brown booby colony on Belmonte Island is composed of over a hundred nests (this study; Kohlrausch 2003),

which are distributed on the sloping stones located at the northwest face of the island (Fig. 1). The colony is heterogeneous regarding landscape features: nests vary in altitude, distance from the sea, susceptibility to waves, and between-nests distance. As SPSP is located on the Equator, it is directly influenced by the seasonality of the Intertropical Convergence Zone, a low pressure area that meets easterly surface winds forming a cloudy band with increased precipitation around the globe (Rihel et al. 1979). From February to May, the Intertropical Convergence Zone is between 7°S e 8°N and, by April, the maximum accumulated precipitation reaches 370 mm on SPSP (Soares et al. 2012). Furthermore, SPSP holds a seamount-like seascape in its surroundings and is influenced primarily by the Equatorial Undercurrent, which flows eastward at ~80 m of depth and slows down when it reaches the archipelago, generating vortices and small-scale upwelling that increase local primary productivity (Araujo and Cintra 2009; Soares et al. 2012).

Nest characterization

All the active and non-active nests (i.e. only containing nest material such as small stones and feathers) were georeferenced by using the Real-Time Kinematic (RTK) method with a pre-processed base point and a rover station (Topcon Positioning Systems, Inc.), which was placed at the central point of each nest. Radio-based communication between base and rover stations provides real-time corrections when triangulating to GPS satellites, so that approximately 20 mm-resolution latitude, longitude and altitude data were obtained for each nest. After getting data for 304 points in 1580 m^2, a three-dimensional elevation map was built with a resolution of 0.19 points.m^{-2}. Additionally, nests were individually numbered in photographs of the colony.

Given the landscape heterogeneity of the colony, nests were classified according to a combination of parameters in order to assign each nest regarding scale of nest quality: global

altitude, distance and altitude in relation to neighbors, distance from the colony edge, and susceptibility to be destroyed by waves (Fig. 2). A cutpoint of 15 m above the sea level (a.s.l.) was established as criterion of quality, so that nests > 15 m were classified as high-quality nests as they are not reached by waves, even during storms. During an extreme event in 2014, all nests below ~15 m were destroyed by waves, representing almost 80% of the active nests (pers. obs.). Colonial breeding has been suggested to be a strategy against predators, so that chick survival seems to be higher in central nesting sites (Forster and Phillips 2009; Minias 2014). Peripheral nests were considered those within a 3 m-range from the outer limit of the colony. Within peripheral nests, there are some nest locations that are usually destroyed by waves, mainly during syzygy tides, which were assigned as low-quality nests. Position in relation to neighbors was defined by ranking altitude of each nest according to the three nearest nests, so that nests in lower places are more susceptible to be flooded by rain, making eggs and chicks soaked. Nests in the peripheral area with the lowest local altitudes were also classified as low-quality nests. For each nest, distances from the three nearest nests were taken to represent average between-nest distance. The average between-nest distance for nests < 15 m of altitude was 1.2 m (see the 'Results' section) and, therefore, nests out of the peripheral area and not being the lowest among neighbors with average between-nest distance > 1.2 m were classified as high-quality nests. All nests that did not fit into the parameters for low and high quality nests were classified as intermediate quality nests.

Sampling scheme

During June 2013, May–June 2014 and June–July 2015, morphometrics and body mass measurements from breeding boobies were obtained after capturing individuals on the nests by hand or handnet. Body mass was obtained to the nearest 20 g using Pesola® spring scales, and morphometrics were measured as follow: culmen length (exposed culmen) and tarsus length

(from middle of the midtarsal joint to the distal end of the tarsometatarsus) with vernier calipers (0.01 mm), and wing chord (carpal joint to the tip of the longest primary; unflattened wing) with a metal rule with stop (1 mm). Gender was determined by differences in skin coloration around the eyes, and adults were distinguished from juveniles by plumage coloration (Nelson 2005). After sampling, each booby was identified with a numbered tarsal metal ring to avoid resampling.

During the fieldwork carried out in 2015, boobies for sampling were divided into subgroups based on body size and, for this purpose, each gender was treated as a distinct dataset, because there is a strong and reverse sexual size dimorphism in brown boobies (Nelson 2005). To generate a global body size index, wing chord, culmen and tarsus length, and body mass were standardized, by subtracting the mean and dividing by the standard deviation, and synthesized through a Principal Component Analysis. The first principal component (PC1), which explained 66.1% of the total variance, was adopted as a body size index and used for sorting individuals. In order to group individuals in relation to body size, males and females PC1 (body size index) were ranked and cut into 20% quantiles: the first quantile (0–20%) was treated as the smallest individuals, the third quantile (40–60%) was treated as intermediate individuals, and the fifth quantile (80–100%) was classified as the largest individuals (Online Resource 1). Because adults in distinct breeding stages were sampled, the non-parametric Kruskal-Wallis test was used to test for an effect of nest content on foraging behavior and diet parameters. Chicks were aged using the classification suggested by Simmons (1967): N1 (0–3 weeks); N2 (4–6 weeks); N3 (7–11 weeks); N4 (12 weeks to fledgling).

Foraging behavior

For studying foraging movements, miniaturized high-resolution GPS, with an integrated chip antenna and rechargeable battery were used (12.5 g, 19 × 25 × 5 mm; Technosmart, Rome,

Italy). Loggers were set to record 1 position every second when deployed for one day, and 1 position every 10 seconds when deployed for two days. During 30 days in July 2015, boobies were captured for logger deployment between 04:00 and 05:00 h, and GPS were attached to the three central rectrices using TESA tape. Loggers were packaged within heat-shrinkable waterproof tube, and the total mass of the equipment (i.e. tube, tape, and logger) did not exceed 3% of the body mass considering the lightest brown booby at SPSP (a male of 1185 g), as recommended for seabirds (Phillips et al. 2003). After logger retrieval, data were downloaded using the dedicated GiPSy-4 Utility software (Technosmart, Rome, Italy). Logger deployment and retrieval (when biological samples were taken) lasted ~5 and 10 minutes, respectively, and in both handling procedures, the bird was returned immediately to its nest in order to minimize stress.

Diet parameters

Diet of boobies was studied during the same aforementioned period of tracking by using stable isotopes of carbon and nitrogen of blood serum along with regurgitated material analysis. For serum isolation, 3-ml blood samples were taken from the tarsal vein during GPS recovery, with sterile syringe/needle and transferred to a non-heparinized tube, which was kept undisturbed at room temperature during 10–30 min for blood clotting. After clotting, tubes were centrifuged during 25 min at 3000 RPM to isolate 1 ml of serum, which was then transferred to 2-ml tubes using clean pipette tips and frozen at -4 °C or lower until processing in the laboratory. Bird serum has isotopic half-life < 5 days (Boecklen et al. 2011) and, thus, carbon and nitrogen isotopic ratios obtained in this study correspond to the period immediately prior to the remote tracking. Spontaneous regurgitation of stomach content is a usual behavior in Sulidae species during stressful situations (Nelson 2005). During handling, non-digested regurgitate material was collected, identified at species level, and measured at fork length using a ruler with 1-mm

accuracy. Additionally, muscle samples from prey items, which have isotopic half-life of approximately 30 days for fish, were taken and stored in anhydrous ethanol for stable isotope analysis (Bugoni et al. 2008).

In laboratory, muscle samples were washed in a Soxhlet extractor during three sessions of 8 h each in order to remove lipids by using a 2:1 chloroform:methanol solution as solvent (Logan and Lutcavage 2008). Muscle and serum samples were freeze-dried, homogenized, and subsamples of ~0.7 mg were transferred into tin capsules to be analyzed in mass spectrometer (measurement precision of 0.2‰) at the Stable Isotope Core, Washington State University (USA). Difference between sample ratio and international reference standards (Vienna Pee Dee Belemnite limestone for carbon, and air for nitrogen) was expressed in δ notation as parts per thousand (‰), which was determined by the equation proposed by Bond and Hobson (2012).

Statistical analyses

Accuracy of GPS positions used for tracking was < 10 m in more than 95% of the location fixes, which made possible to reconstruct foraging trips and to extract high-resolution foraging parameters, such as total distance travelled ('D', km), maximum distance to the colony ('Dmax', km), time spent at sea ('T', h), sinuosity of the path ('Sin', defined as the ratio $D.2Dmax^{-1}$), and average flight speed ('FS', $km.h^{-1}$).

Carbon and nitrogen isotopic ratios were used for calculating isotopic niche width through the Bayesian framework implemented in the SIBER package (Jackson et al. 2011). Standard ellipse areas ($‰^2$) corrected for small sample sizes (SEAc) were estimated for each category of body size along with pairwise overlap percentage between ellipses. Proportions of prey contribution to the diet of each group were estimated with Bayesian mixing models as implemented in the MixSIAR package (Stock and Semmens 2013). For this, isotopic ratios of the three most important prey species found in the %PSIRI analysis (see below) were used as

source data, and trophic enrichment factors were 1.1 ± 0.5 for $\delta^{13}C$ and 2.8 ± 0.5 for $\delta^{15}N$, following an experimental study with a piscivorous seabird and lipid free tissues (Bearhop et al. 2002). Posterior distributions for each prey item for each group were obtained after run 1,000,000 MCMC, discarding the first 500,000 as 'burn-in' (Stock and Semmens 2013).

Characterization of boobies diet from regurgitate material followed the Prey-Specific Index of Relative Importance ('%PSIRI'; Brown et al. 2012), which is based on prey-specific parameters (i.e. ranging from >0% to 100%), such as frequency of occurrence, abundance, and mass or volume. Because prey may be sampled in variable stages of digestion, individual mass was predicted from fork length by using previously published Bayesian regression parameters for elongated-body fish species (Froese et al. 2014).

Differences of body size between categories of nest quality were tested with univariate Analysis of Variance (ANOVA), and normality and homoscedasticity of residuals were tested with Shapiro-Wilk's and Levene's tests, respectively. Intersexual differences regarding foraging trip parameters and isotopic data were tested with the non-parametric Mann-Whitney U test, since there was no normality and homogeneity of variances for residuals. Similarly, intrasexual differences of foraging parameters and stable isotopes were tested with the non-parametric Kruskal-Wallis. As intrasexual relationships between body size and behavioral and diet variables were expected to be non-linear, two distinct approaches were used to test for association of isotopic data and foraging trip parameters with PC1. Firstly, intrasexual differences of foraging behavior and diet were tested with the non-parametric Kruskal-Wallis by using body size groups as factors. The second approach was based on correlations between the continuous body size index and behavioral and diet variables by using the non-parametric Kendall's tau (τ), which measures correlation between two ranked variables (Legendre and Legendre 2012). Finally, regression trees were used to identify the importance of body size in explaining the variance of each foraging and diet parameter. For classifying variables according

to their importance at each regression tree, the Random Forest algorithm implemented in the R package *randomForest* was applied (Liaw and Wiener 2002), by generating 1000 regression trees with 20 per tree permutations for out-of-bag data. Classification was carried out by ranking variables according to the resulting error rate caused when each variable was removed from the model (i.e. the higher error rate, the higher importance).

Results

Global results and intersexual differences

In total, 319 brown boobies (160 females and 159 males) had their biometrics assessed. On average, females were larger and heavier than males: 5.1% for culmen length, 7.1% for tarsus length, 4.4% for wing chord, and 25.3% for body mass (Table 1). From 2014 to 2015, nest fidelity was ~70% (n = 90) and mate fidelity was ~90% (n = 37). Stable isotope values ranged from -17.52 to -16.82‰ for δ^{13}C and from 11.24 to 13.33‰ for δ^{15}N, and females presented significantly higher values for both carbon and nitrogen ($P < 0.05$). SEAc were similar for males (0.22‰^{2}) and females (0.18‰^{2}), and Bayesian ellipses overlapped by 21%. In total, 72 stomach contents and 307 individual prey from 60 distinct individual birds (36 females and 24 males) were analyzed and the asymptote of prey species richness was reached with 21 stomach contents and 55 prey. All the seven prey items were identified at species level, and the tropical two-wing flyingfish *Exocoetus volitans* (hereinafter referred to as 'TTF') was the most important prey item (PSIRI = 62.8%), followed by the bigwing halfbeak *Oxyporhamphus micropterus* (hereinafter referred to as 'BH'; PSIRI = 17.6%) (Table 1) Stable isotopes measured from muscle samples of prey species ranged from -18.28 to -17.31‰ for δ^{13}C and from 7.42 to 11.11‰ for δ^{15}N (Online Resource 2). Due to its high prey-specific importance, TTF's were separated into three food item categories according to fork length, as small (< 100 mm), intermediate (100−150 mm) and large individuals (> 150 mm) for both MixSIAR and subsequent PSIRI analyses. No

substantial between-sex differences were observed from PSIRI analyses, but large TTF had more importance for females, while intermediate TTF and BH were more important for males in comparison to females (Online Resource 3). Mixing models from MixSIAR considering the three most important food items (large and intermediate TTF, and BH) as potential prey presented similar results, so that mean posterior probabilities with 95% confidence interval for females were 69.7% for large TTF, 7.2% for intermediate TTF, and 23.1% for BH, while for males it was 59.1%, 5.9%, and 35%, respectively (Fig. 3; Table 1; Online Resource 4). Foraging behavior was recorded for 97 brown boobies (52 females and 45 males), summing 258 trips. Mean (± 1 standard deviation) trip duration was 57 ± 27.5 min, while maximum distance from the colony and total trip length were 7.1 ± 4.3 km and 27 ± 13.7 km, respectively. No significant intersexual differences were observed regarding foraging trip parameters (Fig. 4). Accordingly, nitrogen and carbon isotopic ratios were the most important variables to discriminate sexes in the regression tree, followed by Dmax, T, D, and Sin, respectively. Nest content had no significant effect on foraging trip parameters and isotopic data for both males and females. Therefore, no individuals were removed from male and female datasets for the subsequent analyses (Online Resource 5).

Nest characteristics

Although 304 nests were sampled for landscape characteristics, only 112 nests were active during the study and, therefore, were classified according to their quality. Colony area considering the minimum convex polygon was 601 m^2, which represents a density of 0.51 $nests.m^{-2}$. Nest altitude ranged from 10.7 to 20.7 m a.s.l., and average between-nest distance was 1.03 ± 0.16 m. Nest altitude was negatively correlated with mean between-nest distance (τ = -0.15; P = 0.0004), so that nest density was larger in high altitude areas. Considering 15 m a.s.l. as a cut point, nest density in areas > 15 m was 0.89 $nests/m^2$ (114 nests in 128.9 m^2),

while it was 0.63 nests.m^{-2} (190 nests in 301 m^2) in areas < 15 m a.s.l. In total, 26.2% of all nests were assigned as low-quality, 29.9% as intermediate, and 43.9% as high-quality nests (Fig. 5).

Intrasexual comparisons

Differences in mean body size of females occupying nests with different quality were detected (F = 9.67; P = 0.0001), so that there was significant difference of mean body size between high and low (P = 0.0002) and high and intermediate quality nests (P = 0.02). Larger females predominated in high quality nests. In contrast, differences in mean body size of males in relation to nest quality were not detected (Fig. 6). No significant differences (*i.e.* P < 0.05) in foraging trip parameters were observed between groups of body size for both males and females, neither was there a significant correlation between the continuous body size index and trip parameters for both genders (Fig. 4; Table 1). In addition, no significant correlation or differences of mean values were observed between body size and δ^{13}C and δ^{15}N values, as well as mean overlapping of Bayesian ellipses between body size groups was 26% for females and 24% for males (Fig. 7). Prey item richness ranged from three to seven species among body size groups of females and males, and TTF was identified as the food item of high prey-specific importance for both genders, corresponding to 79.6%, 90.7% and 80.6% of the diet for small, intermediate and large females, and to 57.4%, 41.7% and 76.3% for small, intermediate and large males, respectively (Table 1). There were no substantial differences in PSIRI results between small, intermediate and large body sizes for both females and males, but comparisons of PSIRI results between body size groups of males had low power due to the small sample size (Table 1). Carbon and nitrogen isotopic ratios were also statistically similar (P > 0.05) between body size groups for males and females, and contributions of potential food items generated by mixing models were similar among groups (Figure 3; Table 1). Finally, the highest importance

of body size was observed in the regression trees explaining variance of nitrogen for males (the third most important) and carbon for females (the second most important), so that body size was not the most important variable in any regression tree (Online Resource 6).

Discussion

Intersexual differences in foraging parameters

Intersexual differences were detected mainly for diet parameters, and higher values of both carbon and nitrogen stable isotopes observed for females could be related mainly to the higher contribution of large prey species. Although the diet of females was 55% represented by large TTF, it was also composed by the fourwing flyingfish *Hirundichthys affinis*, the margined flyingfish *Cheilopogon cyanopterus* and the flying halfbeak *Euleptorhamphus velox*, which had the highest values of carbon and nitrogen and were the largest prey in relation to fork length. In turn, lower isotopic values observed for males could be associated with an increased ingestion of alternative prey, such as the bigwing halfbeak, the intermediate TTF and the bluntnose flyingfish *Prognichthys gibbifrons*, which have lower body size and isotopic values.

The increased importance of larger prey in regurgitate material and higher carbon and nitrogen isotopic values observed in female serum in this study could be attributed to the intersexual body size differences, suggesting trophic niche partitioning as a function of the marked reversed sexual size dimorphism in brown boobies. Niche segregation has been suggested to be involved in the evolution and maintenance of sexual size dimorphism in seabirds, so that dimorphic pairs could avoid competition and enhance feeding efficiency (Serrano-Meneses and Székely 2006). Sulidae species tend to present intersexual differences in foraging parameters, as it has been widely demonstrated for boobies breeding in tropical areas, such as for prey item length (Zavalaga et al. 2007), carbon and nitrogen isotopic ratios (Young et al. 2010), foraging range (Weimerskirch et al. 2006) and dive depth (Sommerfeld et al. 2013).

However, previously published comparisons demonstrate there is no clear pattern in sex-biased behaviors.

For brown boobies, fine inter-colony and temporal environmental heterogeneity seems to influence intersexual differences of diet and foraging behavior rather than body size. For example, female brown boobies from Isla San Ildefonso, Gulf of California, performed significantly larger and longer trips than males (Weimerskirch et al. 2009), while females from Palmyra Atoll, tropical Pacific Ocean, performed shorter trips and with lower duration than males (Lewis et al. 2005). For brown boobies from two other colonies in the Gulf of California, intersexual foraging behavior and dietary differences were minimal through the years and the strong interannual fluctuation in prey availability made clear its potential plasticity following temporal changes. We found no significant behavioral sex differences during foraging trips of brown boobies in SPSP, which could be attributed to the spatially limited prey availability around the archipelago. Nonetheless, this small-scale spatial distribution of flying fishes and boobies in SPSP could be masking fine behavioral differences, not detected by the dataloggers we have used, explaining the slight intersexual differences observed for isotopic data and regurgitate material. Therefore, it is likely that it is not a single selective pressure which maintains the marked reversed SSD observed in Sulidae, but a combination of sex-specific functions related to foraging (e.g. widening of the trophic niche explored by the species) and breeding parameters (e.g. labour division in parental care) making intersexual body size difference an advantageous attribute.

Despite the slight intersexual differences in relation to diet parameters, Exocoetidae species represented more than 95% of the material regurgitate by brown boobies as a whole, shedding light on the importance of this family to sustain a diverse epipelagic community around a tropical remote archipelago. Flyingfishes play a central role to make SPSP an important site for the tropical Atlantic Ocean epipelagic food web, as they spawn around the

archipelago and represent approximately 80% of the fish larvae found in its surroundings during the northern summer, wherein 73% is composed by the margined flyingfish (Lessa et al. 1999; Macedo-Soares et al. 2012). Interestingly, the margined flyingfish, a 330 mm-length species (Monteiro et al. 1998), has been demonstrated to be the most important prey species for wahoo *Acanthocybium solandri*, yellowfin tuna *Thunus albacares* (Vaske-Jr. et al. 2003), oilfish *Ruvettus pretiosus* (Viana et al. 2012) and brown booby (Both and Freitas 2001) in the SPSP, but in this study the margined flyingfish did not occur in regurgitates of males and was only rare in female diet. In turn, TTF is not considered as an important prey for these top predators and BH appears only as secondary in importance for seabirds, but in our study TTF was dominant in regurgitate material of both male and female brown boobies. The margined flyingfish is abundant around SPSP from November to April, when it is believed to spawn and use rocks for egg attachment (Monteiro et al. 1998). Indeed, the highest capture rates of pelagic fishes around SPSP are in this period, mainly by the explotation of yellowfin tuna, which is attracted by high concentrations of margined flyingfishes, while the lowest capture rates are observed during the northern winter, when this study was carried out (Vaske-Jr. et al. 2003; Viana et al. 2015). Therefore, we suggest that flyingfishes support breeding activities of brown boobies in SPSP, but with the main prey species alternating between margined flying fish during the northern summer (Both and Freitas 2001) and TTF during the northern winter (as found here), demonstrating the high primary productivity fueling the food web in SPSP throughout the year, despite this being a tropical remote archipelago surrounded by oligotrophic waters.

Colony landscape selecting phenotypes

No substantial associations of diet and foraging trip parameters with body size of males and females were observed, but significant difference of mean body size was detected between

females nesting in low and high quality nests. Brown boobies breeding in SPSP have marked intersexual differences in nest attendance, so that females dedicate significantly more time to incubation, brooding, and chick provisioning (Kohlrausch 2003). Furthermore, nest defense is also female-biased in boobies from SPSP (Kohlrausch 2003), what could be critical to ensure egg or chick safety and avoid extreme injuries, such as chick predation by cannibalism (Neves et al. 2015). Considering the high colony density we found in SPSP, we suggest that spatial constraints for nesting are the major driver for shaping fitness traits in brown boobies, positively selecting large females, which would benefit from their body size during fights for high-quality territory conquest and defense.

Interestingly, the normal distribution curve of female body size was more acute than that of males, but slightly skewed towards large females, suggesting decreased phenotypic plasticity and reinforcing the idea of positive selection on intermediate and large females. The parameters we used to qualify nests are closely related to breeding success in other colonial bird species (e.g. Thompson and Furness 1991; Svagelj and Quintana 2011; Minias 2014) and, therefore, predominance of small females in low-quality nests suggests a negative selection on this group. Indeed, mean body size and mass of brown boobies from SPSP are not explained by ecogeographical rules and are the highest among brown boobies breeding along the southwest Atlantic Ocean (Nunes et al. 2017), suggesting higher breeding success of large and intermediate individuals in comparison to small ones, and increasing the average body size of birds in this particular population. Furthermore, the population is genetically distant from adjacent populations (i.e. Rocas Atoll and Fernando de Noronha Archipelago), what could be explained by the adaptation to local seascape causing phenotypic differentiation and, consequently, selection against immigrants (Nunes and Bugoni, in review).

As for the intersexual comparisons, absence of intrasexual differences in foraging behavior could be associated to the abundant food availability around SPSP. Brown boobies

from the Gulf of California spent ~2.5 h at sea during foraging trips and travelled almost 80 km on average, reaching 28 km from the colony (Weimerskirch et al. 2009). In the Caribbean, brown boobies spent ~6 h at sea during foraging trips, travelling 129 km and reaching 48 km from the colony (Soanes et al. 2015). The relatively small foraging range of brown boobies around SPSP, revealed by the short mean trip duration (0.85 h), mean total distance (26 km) and mean maximum distance from colony (7 km), suggests there is plenty of food available in the immediate surroundings of the archipelago. This is consistent to the seamount-like oceanographic dynamics observed around SPSP, what enhances local primary productivity on a very small spatial scale due to the interaction of the Equatorial Undercurrent with the submarine relief, increasing residence time of nutrients and generating vortices and small-scale upwelling in the surroundings of the archipelago (Soares et al. 2012; Souza et al. 2013). The abundant food availability close to the colony suggests that there is no segregation between body size groups in relation to foraging behavior for boobies from SPSP, once prey seems to be easily accessible for all. Although individual differences in foraging trip parameters in relation to body size have been demonstrated for other booby species (Lewis et al. 2005; Sommerfeld et al. 2013), obtaining food seems not to be the main selection pressure on phenotypes of boobies from SPSP.

Studies addressing fine individual differences could benefit from research tools with even higher resolution and information provided by long-term monitoring programs. We are convinced that there are individual covariates which were not sampled due to logistic constraints that could affect the findings, for instance personality (Gosling and John 1999), senescence (Reed et al. 2008), and early body condition (Barbraud and Chastel 1999). Additionally, dataloggers capable to detect behavior in higher resolution, such as accelerometers and time-depth recorders, could provide valuable information on underwater

activities (e.g. Cox et al. 2016) and daily energy expenditure (e.g. Elliott et al. 2013), for example.

Nonetheless, our findings demonstrate that available area in terrestrial environments could be a relevant limiting factor even for strictly marine vertebrates, such as seabirds. This is particularly relevant for seabird conservation, as habitat degradation and human disturbance in colonies have been attributed as the main threats for seabirds globally (Croxall et al. 2012) and, thus, decreasing available area for nesting could make territory disputes even more fierce favoring individuals capable to breed in high quality nests. Other marine vertebrate species depending on land for reproduction activities could also be affected by the individual ability to obtain high quality breeding locations. For example, grey seals *Halichoerus grypus* breeding on sand dunes were demonstrated not to have breeding site fidelity due to the changes and unpredictability of colony landscape (Weitzman et al. 2017), but grey seals breeding on rocky islands have shown colony and breeding location fidelity, suggesting that colony landscape heterogeneity promotes inter-individual disputes for high quality breeding areas (Pomeroy et al. 2000). Additionally, climate change can strongly affect ice-dependent species by impoverishing ice conditions required for breeding, increasing young mortality and frequency of sabbatical years, and forcing dispersal to more suitable breeding areas (Stenson and Hammill 2014; Chambert et al. 2015). Over the past 100 years, global mean sea level rose by 0.19 and is expected to increase ~0.5 m by 2100 compared to the current level (IPCC 2014). From this, breeding activities of boobies nesting in the lowest areas in SPSP will be even more difficult than it currently is, suggesting population decline due to reduction of suitable areas for nesting, and a trend of increasing average body size due to the intensification of disputes for high quality nests. In this context, understanding current limiting factors, individual quality and population plasticity is crucial to predict how natural populations will respond to rapid environmental changes promoted by human disturbance in colonies, habitat degradation, and global warming.

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Table 1 Data obtained (mean ± 1 standard deviation) from brown boobies *Sula leucogaster* breeding in the Saint Peter and Saint Paul Archipelago, in the Tropical Atlantic Ocean. Phenotypic data correspond to morphometrics and body mass. Diet contribution (%) was obtained from regurgitate material after calculating Prey-Specific Index of Relative Importance (PSIRI) and from blood serum after stable isotope analyses (± 95% confidence interval). Foraging behavior was estimated by converting spatial data obtained with GPS dataloggers.

	♀				♂			
	Global	Small	Intermediate	Large	Global	Small	Intermediate	Large
Phenotypic data	(*n* = 160)	(*n* = 33)	(*n* = 32)	(*n* = 33)	(n = 159)	(*n* = 33)	(*n* = 33)	*n* = 33
Culmen length (mm)	108.2 ± 2.6	104.8 ± 1.8	107.3 ± 2.3	110.3 ± 1.7	103.2 ± 2.3	101.9 ± 2.1	102.83 ± 1.9	105.1 ± 1.9
Tarsus length (mm)	51.2 ± 1.9	48.3 ± 2.2	51.1 ± 0.9	52.8 ± 1.2	47.9 ± 1.4	45.9 ± 0.9	48.1 ± 0.9	49.2 ± 0.9
Wing chord (mm)	430.7 ± 7.4	423.1 ± 6.7	428.9 ± 6.8	435.8 ± 5.6	412.9 ± 7.2	405.4 ± 3.8	412.8 ± 5.6	418.5 ± 5.1
Body mass (g)	1834.5 ± 227.3	1581.3 ± 176.7	1777.1 ± 181.2	2032 ± 183.3	1545.5 188.9	1388.9 ± 190.2	1483.0 ± 154.6	1682.1 178.4
Regurgitate material (%)	(*n* = 44)	(*n* = 12)	(*n* = 7)	(*n* = 11)	(*n* = 27)	(*n* = 6)	(*n* = 2)	(*n* = 2)
Exocoetus volitans	66.2	79.6	90.7	80.6	59.3	57.4	41.6	76.3
E. volitans (large)	55.5	68.4	80.0	67.0	33.6	22.9	41.6	16.5
E. volitans (intermediate)	10.1	9.7	10.8	13.6	22.2	26.5	0.0	53.7
E. volitans (small)	0.6	1.5	0.0	0.0	3.5	8.0	0.0	6.0
Hirundichthys affinis	9.5	0.0	10.7	0.0	4.3	9.1	30.8	0.0
Oxyphorhampus micropterus	13.2	12.5	9.8	1.9	23.8	19.3	27.5	23.7
Cheilopogon cyanopterus	2.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Prognichthys gibbifrons	4.3	0.0	0.0	3.9	11.9	7.6	0.0	0.0
Euleptorhamphus velox	2.9	0.0	0.0	9.7	1.5	6.6	0.0	0.0
Ommastrephes bartramii	2.5	0.0	0.5	3.8	0.9	0.0	0.0	0.0
Stable isotopes (%)	(*n* = 42)	(*n* = 11)	(*n* = 11)	(*n* = 20)	(*n* = 40)	(*n* = 19)	(*n* = 10)	(*n* = 11)
E. volitans (large)	87.3 ± 0.2	88.5 ± 0.2	87.5 ± 0.2	87.7 ± 0.2	75.3 ± 0.2	75.4 ± 0.2	80.1 ± 0.2	73.9 ± 0.2
E. volitans (intermediate)	3.1 ± 0.1	2.9 ± 0.1	2.9 ± 0.1	2.8 ± 0.1	3.2 ± 0.1	2.6 ± 0.1	2.0 ± 0.1	3.1 ± 0.1
Oxyphorhampus micropterus	9.6 ± 0.2	8.6 ± 0.2	9.7 ± 0.2	9.5 ± 0.2	21.4 ± 0.2	21.9 ± 0.2	17.9 ± 0.2	23.0 ± 0.2
Foraging behavior	(*n* = 52; 144 trips)	(*n* = 11; 62 trips)	(*n* = 14; 43 trips)	(*n* = 22; 42 trips)	(*n* = 45; 114 trips)	(*n* = 19; 57 trips)	(*n* = 10; 31 trips)	(*n* = 11; 34 trips)
Total trip duration (h)	0.9 ± 0.5	0.8 ± 0.4	0.9 ± 0.4	1.1 ± 0.6	0.9 ± 0.4	0.9 ± 0.4	0.8 ± 0.3	1.1 ± 0.4
Foraging range (km)	7.2 ± 3.9	6.8 ± 4.4	7.0 ± 2.7	7.7 ± 4.3	7.1 ± 4.8	6.5 ± 2.2	4.9 ± 2.5	8.4 ± 5.8
Distance covered (km)	27.0 ± 13.2	25.3 ± 13.5	24.4 ± 8.4	29.0 ± 15.4	27.1 ± 14.3	25.4 ± 11.5	21.1 ± 7.1	30.9 ± 14.2
Sinuosity	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	0.5 ± 0.1
Average flight speed (km h^{-1})	29.4 ± 5.9	31.2 ± 3.5	29.4 ± 7.2	30.0 ± 5.4	28.1 ± 5.4	28.4 ± 4.7	25.8 ± 6.3	27.2 ± 4.3

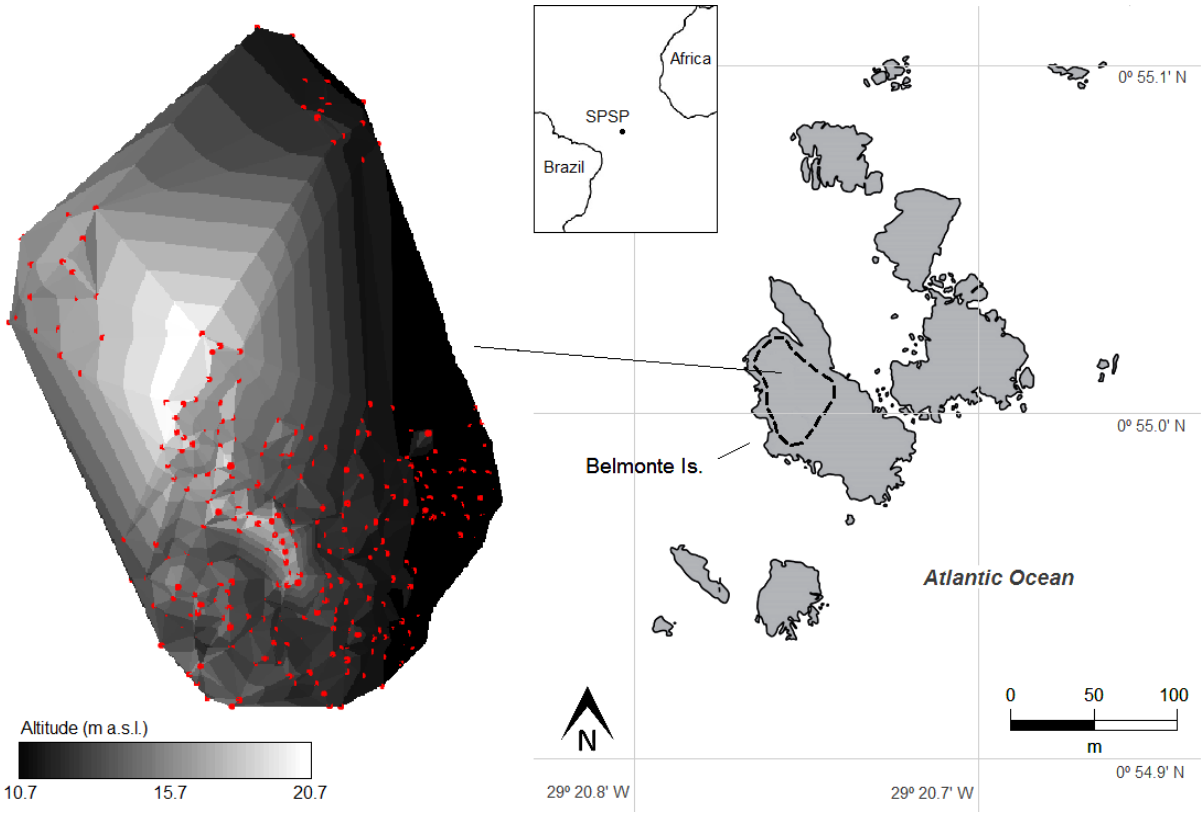

Figure 1 Schematic map of the study area in the Saint Peter and Saint Paul Archipelago. Sampling was carried out in the Belmonte Island, where there is a dense colony of brown boobies *Sula leucogaster* with breeding activities throughout the year (highlighted area). All the 304 active and non-active nests (red points) of the Belmonte Island were sampled for latitude, longitude, and altitude with 20 mm-resolution, which were used to build isolines of altitude for the colony

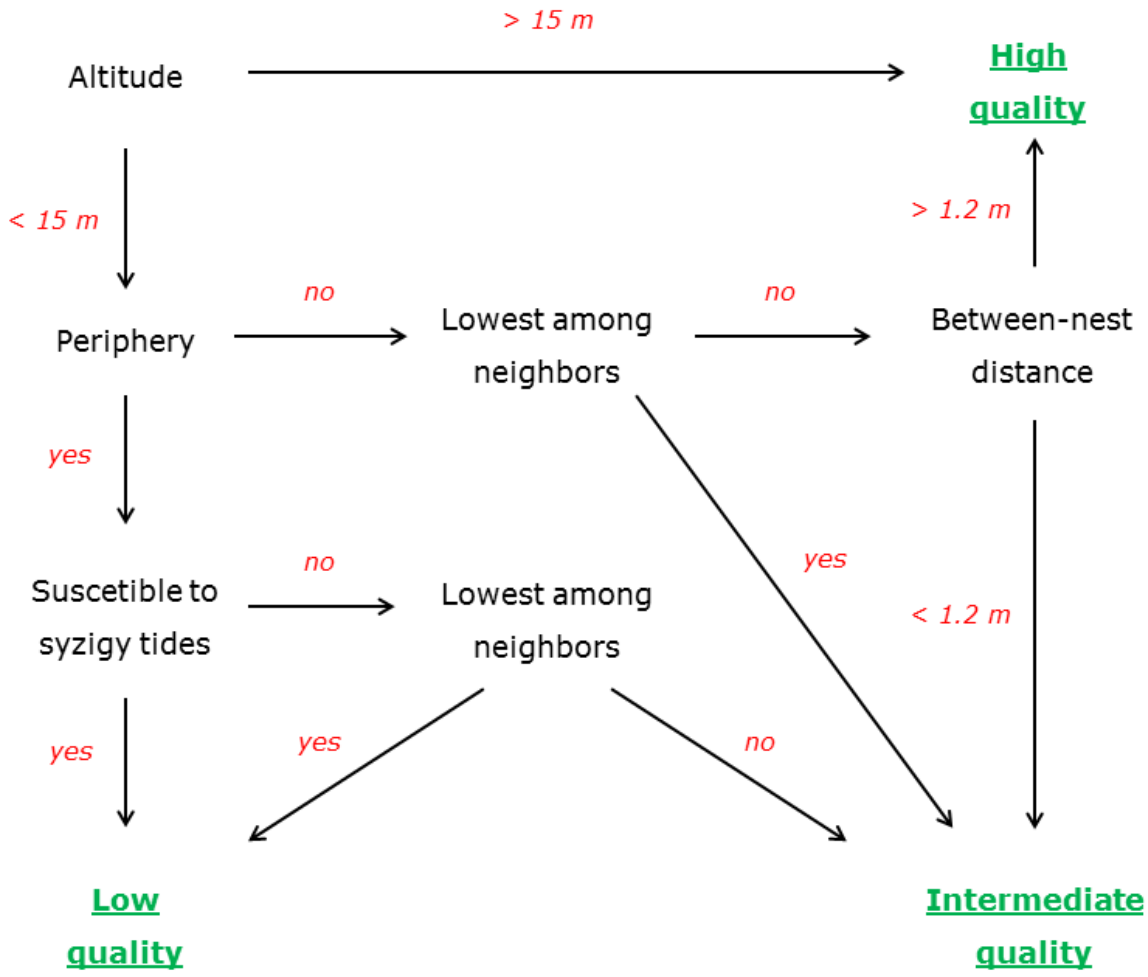

Figure 2 Scheme for classification of nests of brown boobies *Sula leucogaster* breeding in a heterogeneous landscape in the Saint Peter and Saint Paul Archipelago. A cutpoint of 15 m a.s.l. was established, so that nests > 15 m are protected from waves, even during storms. Peripheral nests were regarded as such when placed within a 3 m-range from the outer limit of the colony. Position in relation to neighbors and between-nest distance were defined by comparing altitude and distance, respectively, of each nest according the three nearest nests. The average between-nest distance for nests < 15 m was 1.2 m

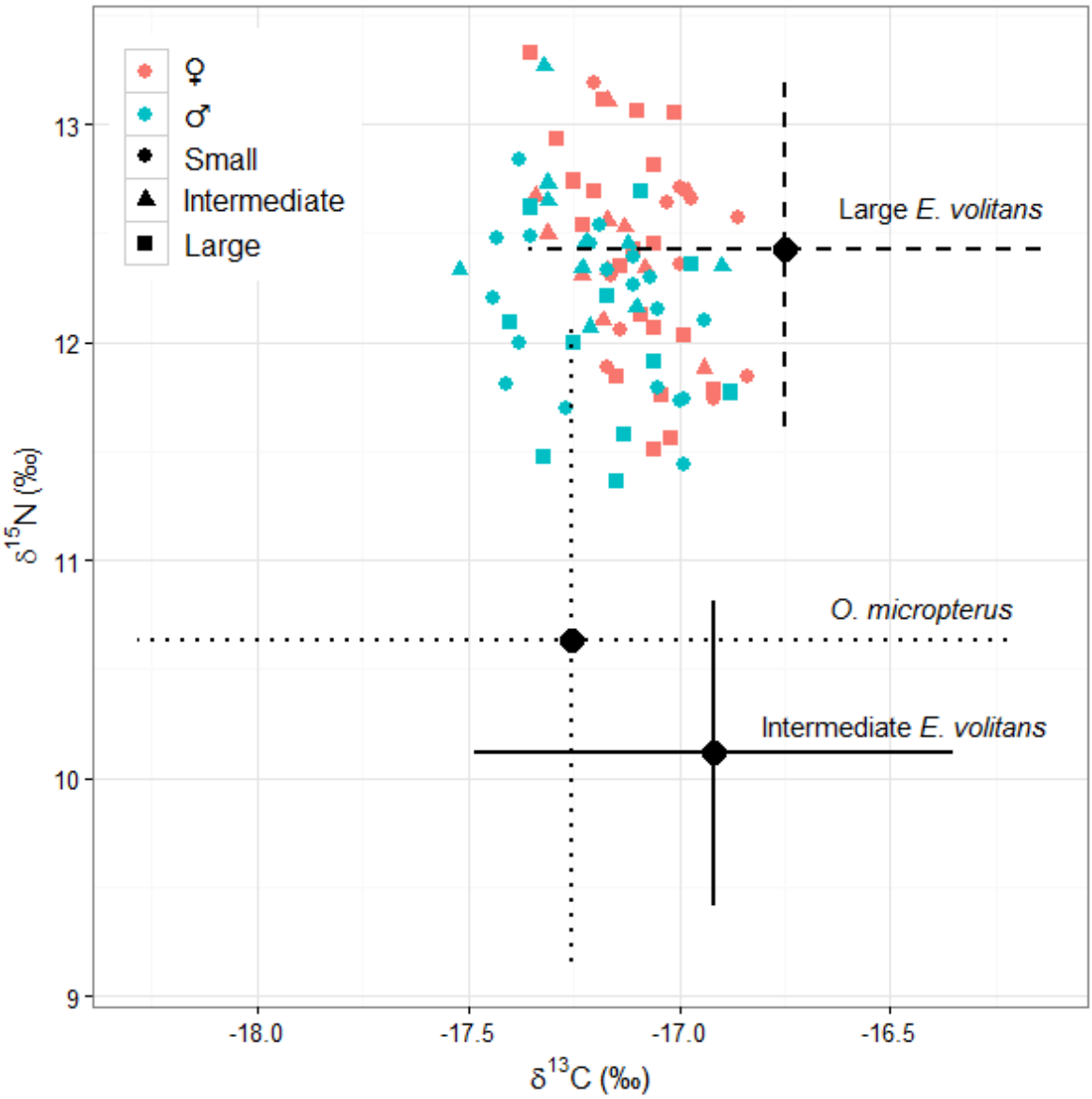

Figure 3 Isospace representing carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopic ratios obtained blood serum of brown boobies *Sula leucogaster* breeding in the Saint Peter and Saint Paul Archipelago and from muscle samples of flying fishes. Brown boobies were grouped according to gender and body size. Prey items are: *Oxyporhamphus micropterus*; intermediate *Exocoetus volitans* = 100-150 mm fork length; and large *E. volitans* > 150 mm fork length. Sampling was carried out in July 2015

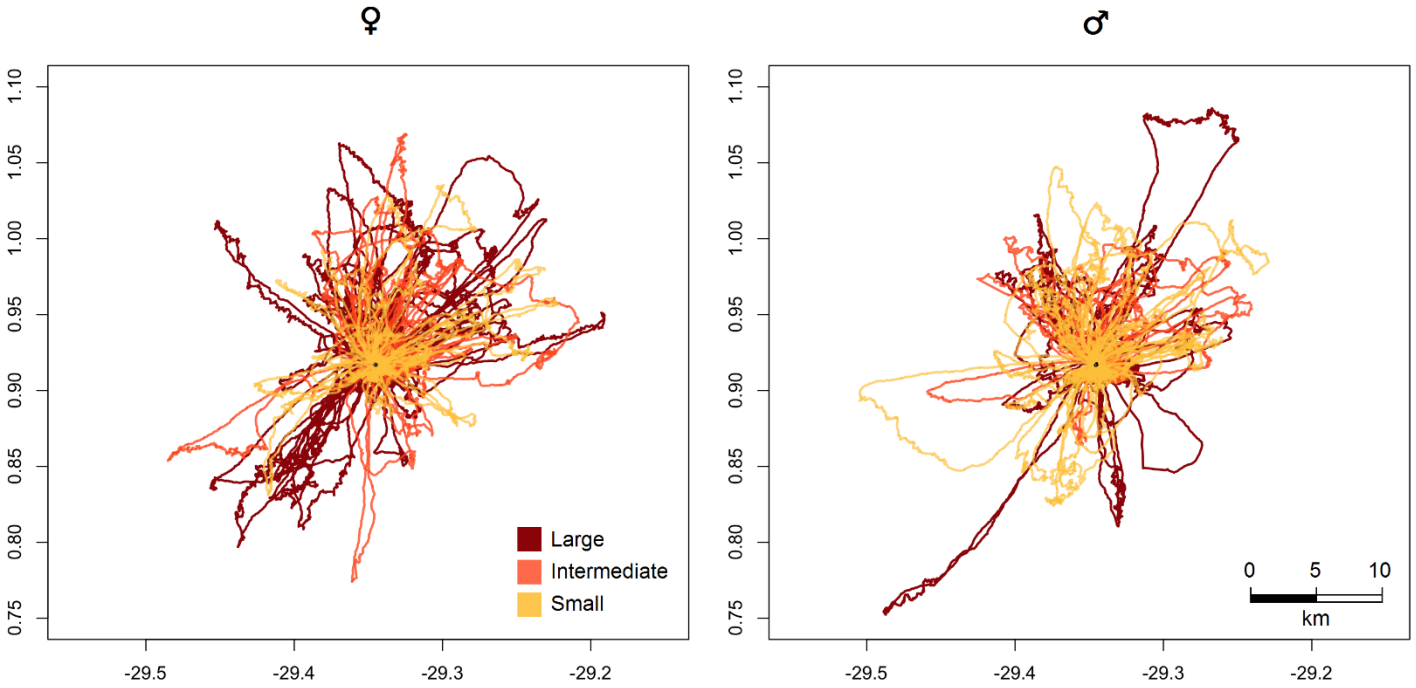

Figure 4 Foraging movements of 97 breeding brown boobies *Sula leucogaster* around the Saint Peter and Saint Paul Archipelago, recorded by GPS at 1 s and 10 s intervals, in July 2015. In total, 144 foraging trips of 52 females and 114 foraging trips from 45 males were recorded

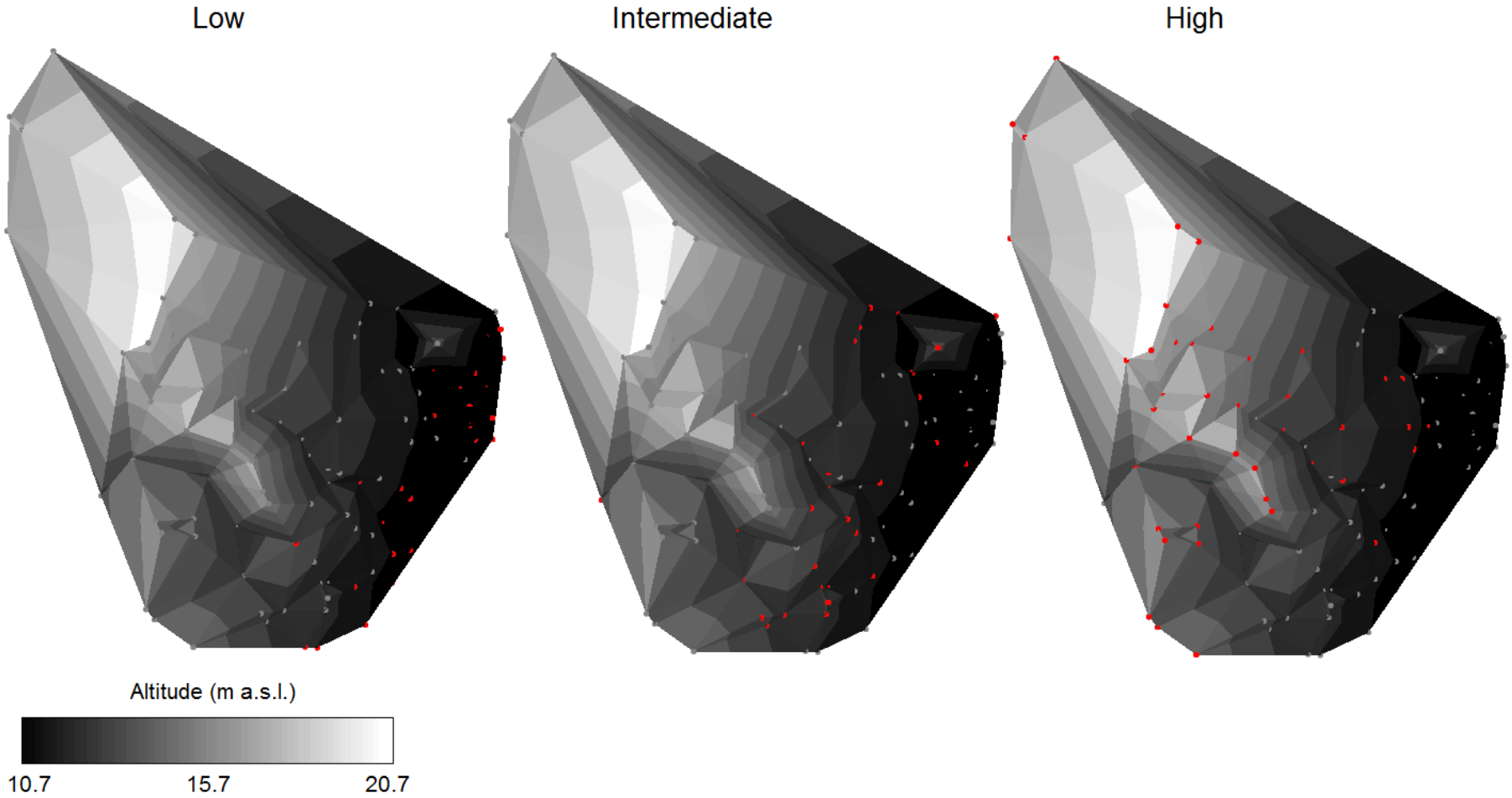

Figure 5 Locations of the 112 active nests (red points) of brown boobies *Sula leucogaster* in the Saint Peter and Saint Paul Archipelago in May–June 2014 and July 2015. Nests were classified as low, intermediate, and high quality, following a scheme based on colony features (Fig. 2)

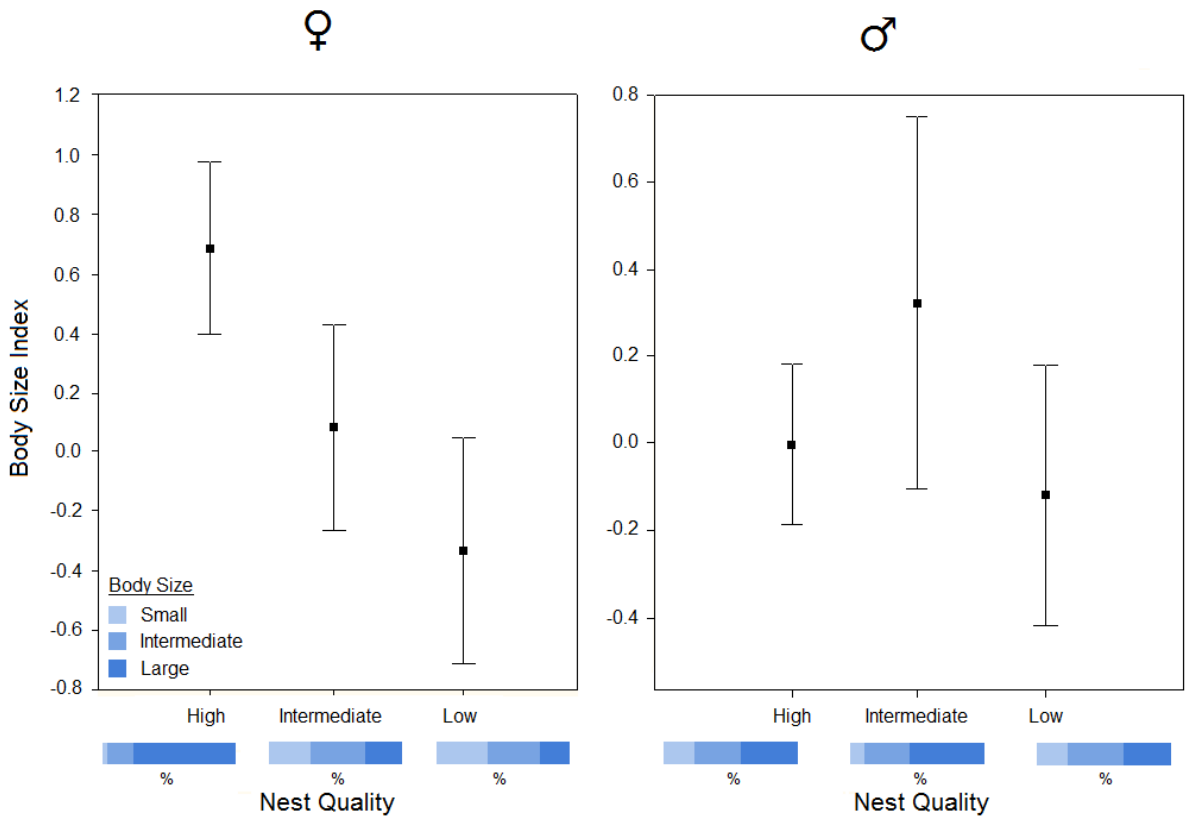

Figure 6 Distribution of body size of male and female brown boobies *Sula leucogaster* breeding in the Saint Peter and Saint Paul Archipelago for each category of nest quality, demonstrated by means and ± 95% confidence intervals error bars. Differences were significant ($P < 0.001$) only for females between high-low and high-intermediate nest quality. Body Size Index is the first principal component (PC1), which was calculated with standardized culmen length, tarsus length, wing chord, and body mass, and explained 66.1% of the variance. Blue bars are showing percentage of each body size group in each category of nest quality

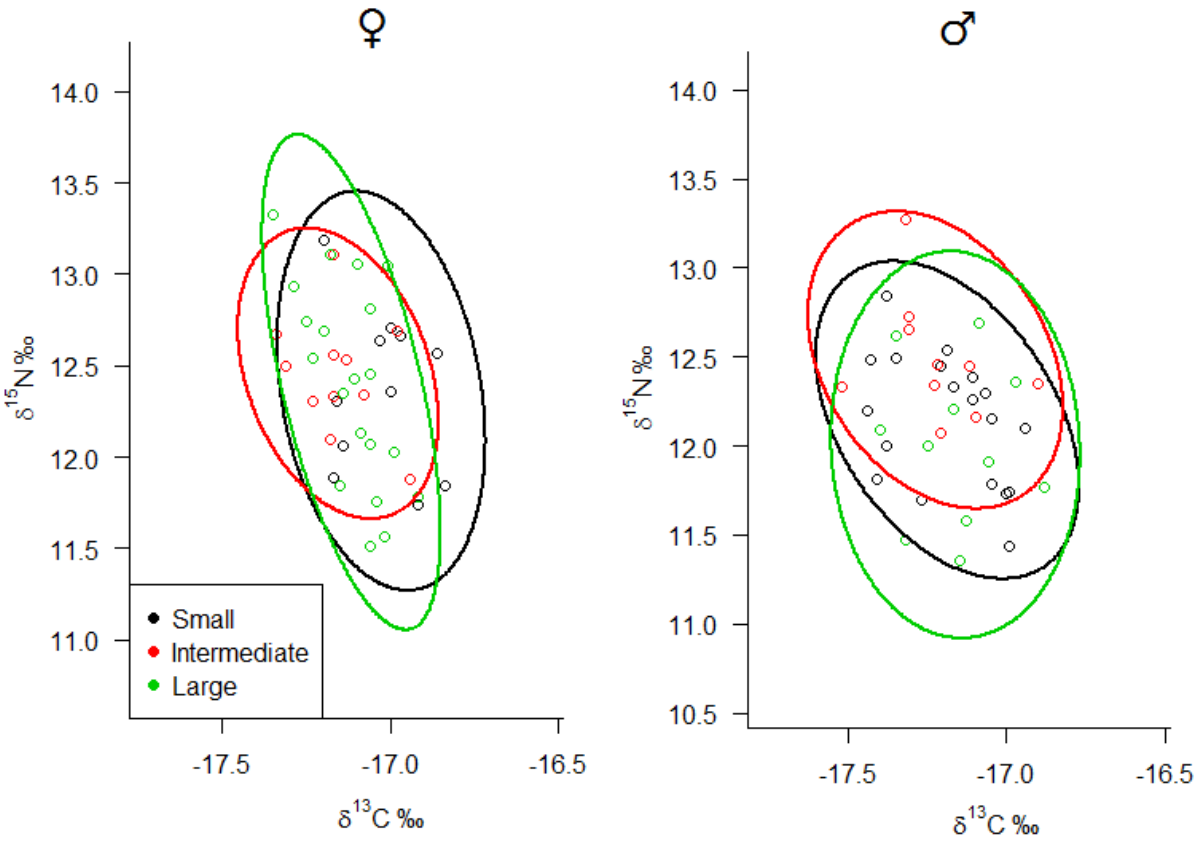

Figure 7 Standard Bayesian ellipses generated with carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic ratios from blood serum of brown boobies *Sula leucogaster* breeding in the Saint Peter and Saint Paul Archipelago. Brown boobies were grouped according to gender and body size. Ellipses comprise 95% of the data

Electronic Supplemental Material (*Oecologia*)

Seabirds fighting for land: fitness consequences of breeding area constraints at a small remote archipelago

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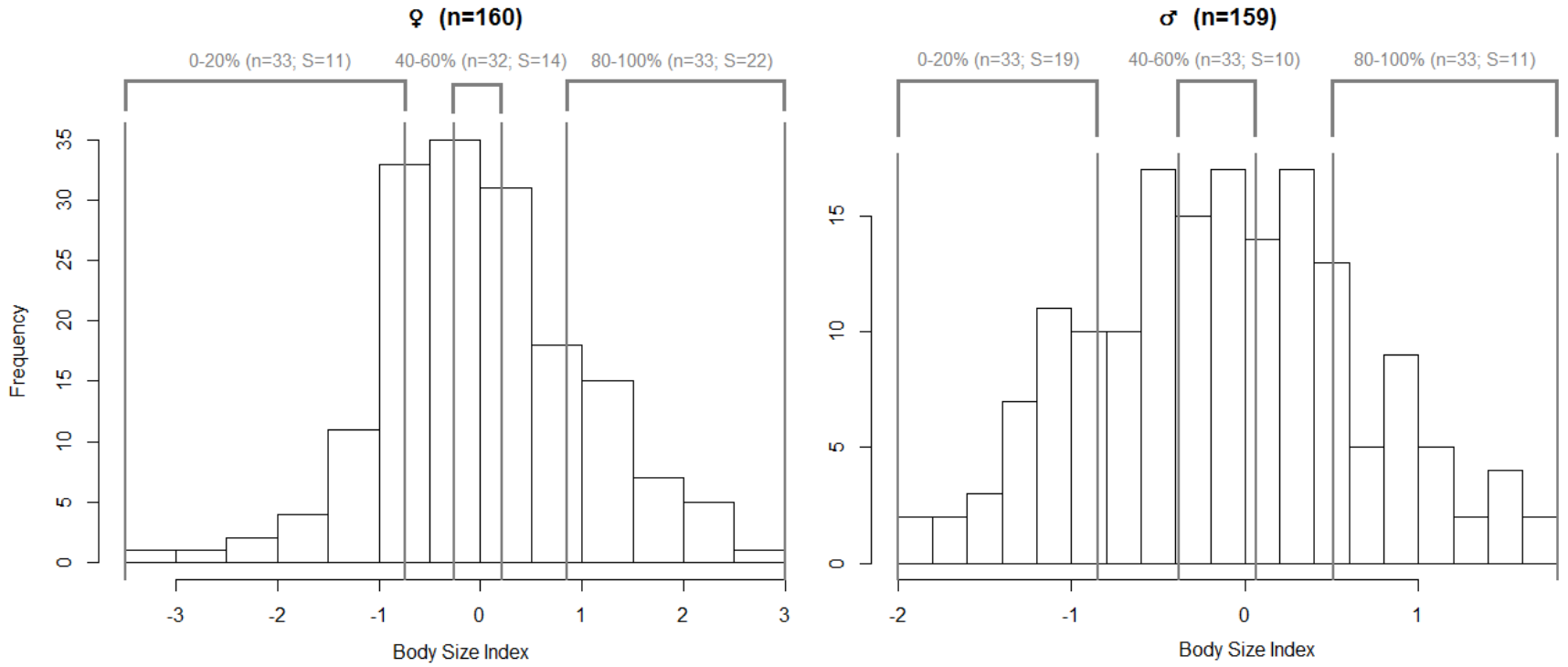
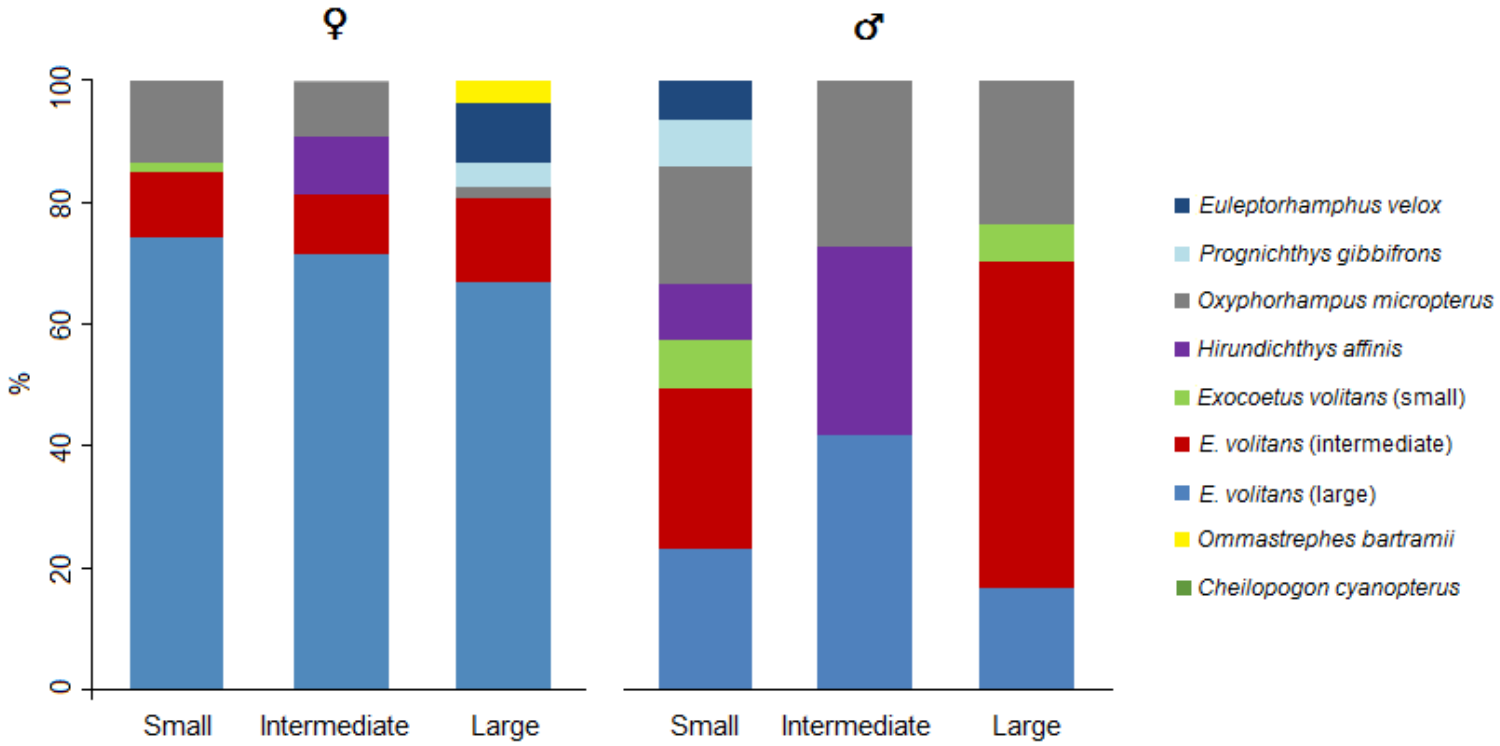


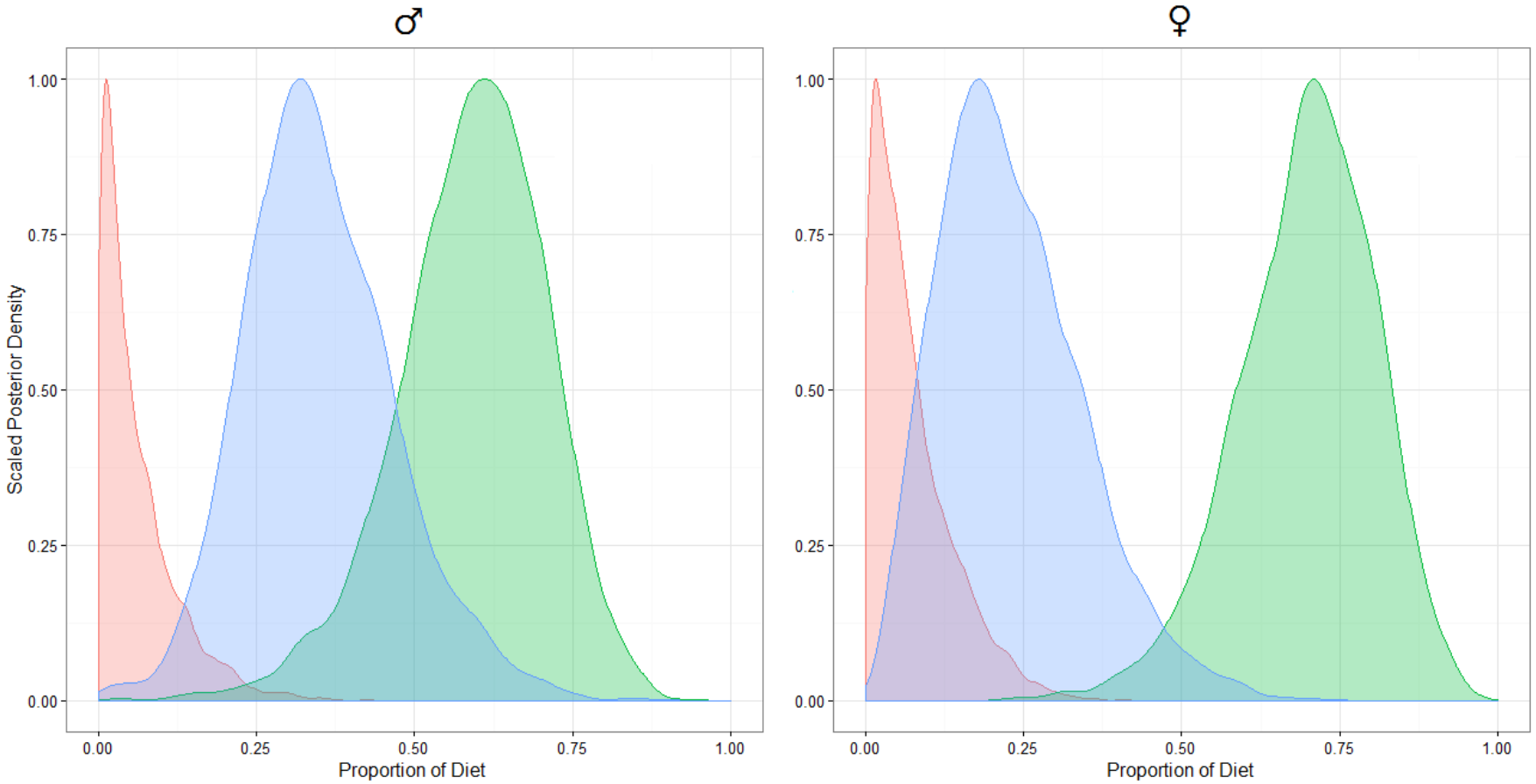
Online Resource 1 Distribution of the Body Size Index for female and male brown boobies *Sula leucogaster* breeding in the Saint Peter and Saint Paul Archipelago. Body Size Index represents culmen length, tarsus length, wing chord, and body mass data converted in a principal component (PC1), which explained 66.1% of the total variance. Individuals were divided into body size groups by defining cutpoints between quantiles of 20%, so that the first quantile (0–20%) was treated as the smallest individuals, the third quantile (40–60%) was treated as intermediate individuals, and the fifth quantile (80–100%) was classified as the largest individuals. n represents the number of individuals in each group, and S represents the number of individuals sampled for diet and foraging behavior parameters

Online Resource 2 Carbon and nitrogen isotopic ratios (mean ± 1 standard deviation) from muscle samples of prey items of brown boobies breeding in the Saint Peter and Saint Paul Archipelago, in July 2015

Prey species	**δ^{13}C (‰)**	**δ^{15}N (‰)**
Exocoetus volitans (n=24)	-17.85 ± 0.62	8.66 ± 1.35
E. volitans (large) (n=6)	-17.71 ± 0.56	9.23 ± 1.16
E. volitans (intermediate) (n=13)	-18.10 ± 0.40	7.42 ± 0.61
E. volitans (small) (n=5)	-18.28 ± 0.51	8.98 ± 0.53
Hirundichthys affinis (n=5)	-17.32 ± 0.49	9.83 ± 1.30
Oxyphorhampus micropterus (n=8)	-17.81 ± 0.67	8.90 ± 1.43
Cheilopogon cyanopterus (n=2)	-17.31 ± 0.03	11.11 ± 0.51
Prognichthys gibbifrons (n=6)	-17.59 ± 0.36	9.19 ± 1.19
Euleptorhamphus velox (n=3)	-17.31 ± 0.52	9.68 ± 1.35
Ommastrephes bartramii (n=5)	-17.93 ± 0.27	9.21 ± 0.26



Online Resource 3 Prey-Specific Index of Relative Importance ('%PSIRI'; Brown et al. 2012) for each prey item found in regurgitate material of small, intermediate, and large brown boobies *Sula leucogaster* breeding in the Saint Peter and Sain Paul Archipelago, in July 2015. In total, 72 stomach contents and 307 individual prey from 60 distinct individual birds (36 females and 24 males) were analyzed. The asymptote of prey species richness was reached with 21 stomach contents and 55 prey. Due to its high prey-specific importance, *E. volitans* were separated into three food item categories according to fork length: small (< 100 mm), intermediate (100−150 mm) and large individuals (> 150 mm)



Online Resource 4 Posterior probabilities with 95% confidence intervals of intermediate *Exocoetus volitans* (red), large *E. volitans* (green), and *Oxyphorhampus micropterus* (blue) used by female and male brown boobies in the Saint Peter and Sain Paul Archipelago (July 2015), estimated with Bayesian mixing models with carbon and nitrogen isotopic ratios obtained from muscle samples of preys and blood serum of brown boobies

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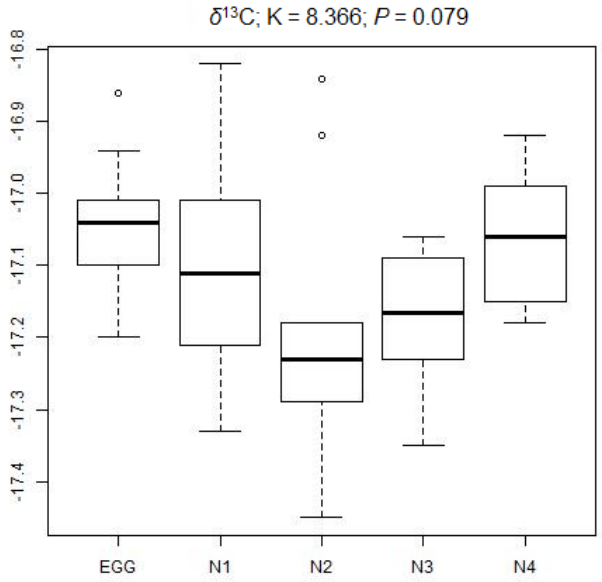
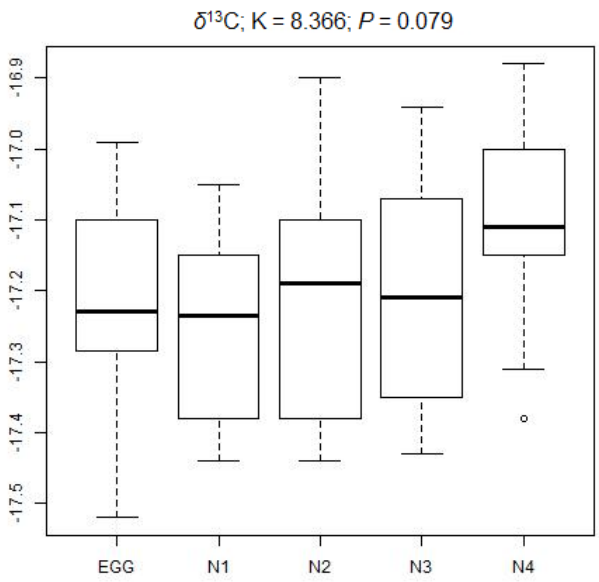

δ13C; K = 8.366; P = 0.079
-16.8
-16.9
-17.0
-17.1
-17.2
-17.3
-17.4
EGG
N1
N2
N3
N4

♂


δ13C; K = 8.366; P = 0.079
-16.9
-17.0
-17.1
-17.2
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-17.5
EGG
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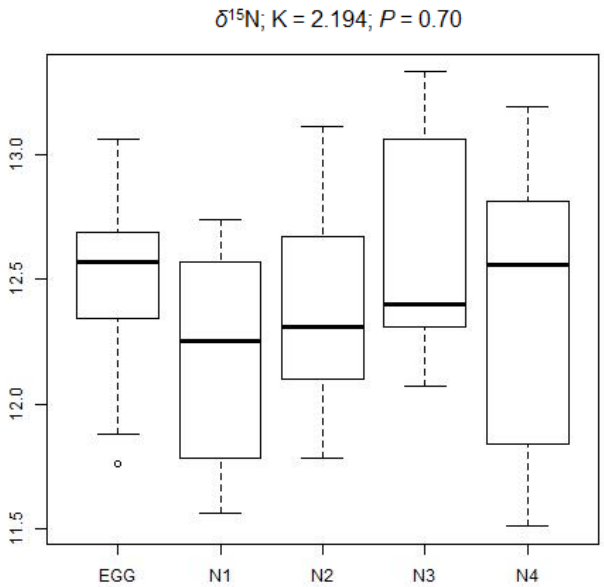
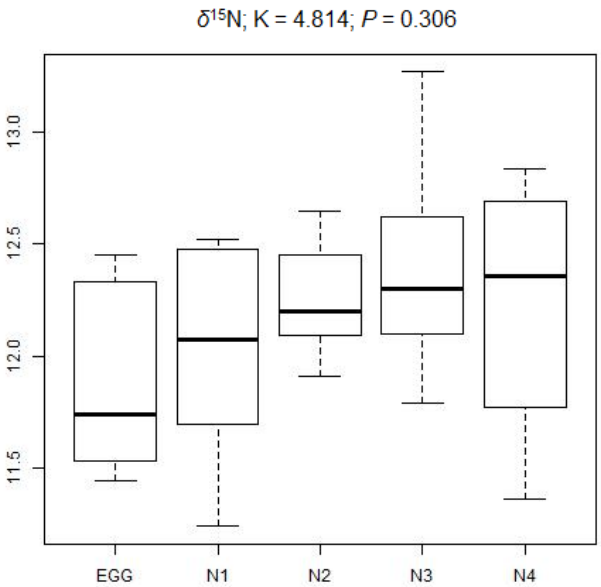
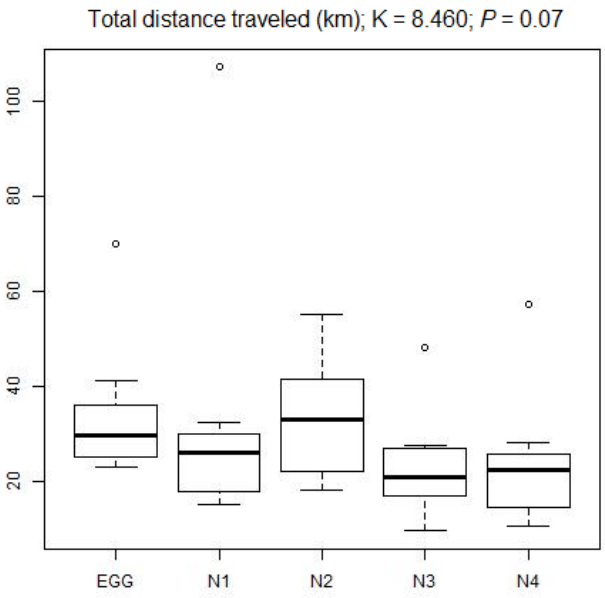
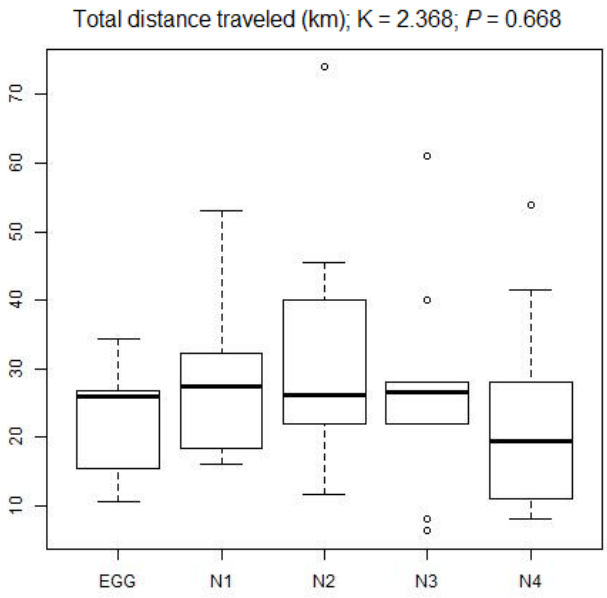
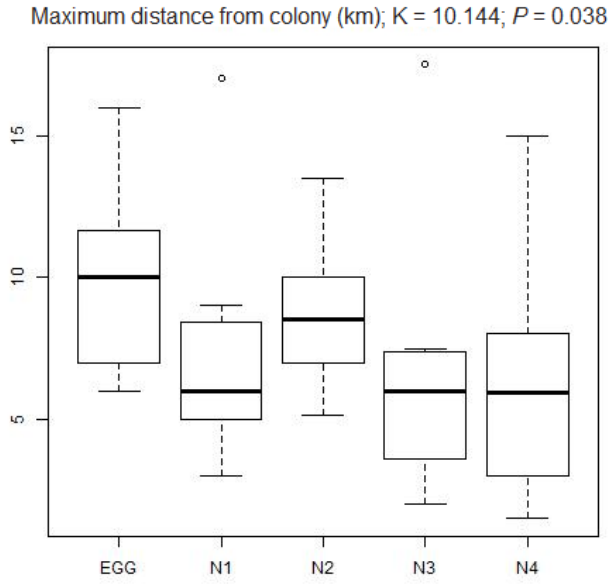
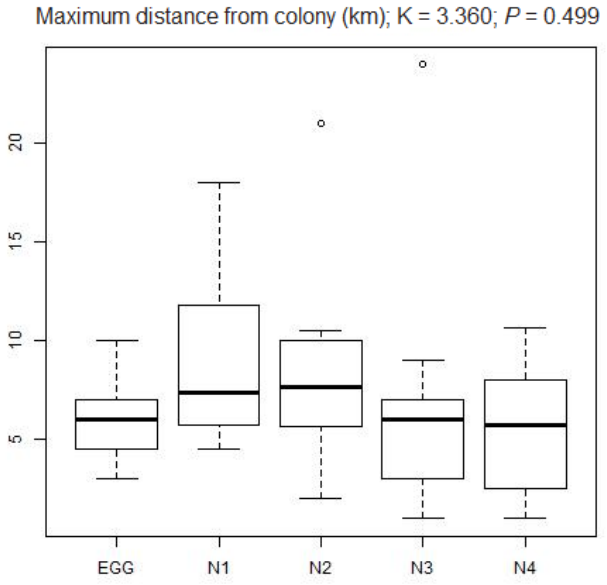

δ15N; K = 2.194; P = 0.70
13.0
12.5
12.0
11.5
EGG
N1
N2
N3
N4

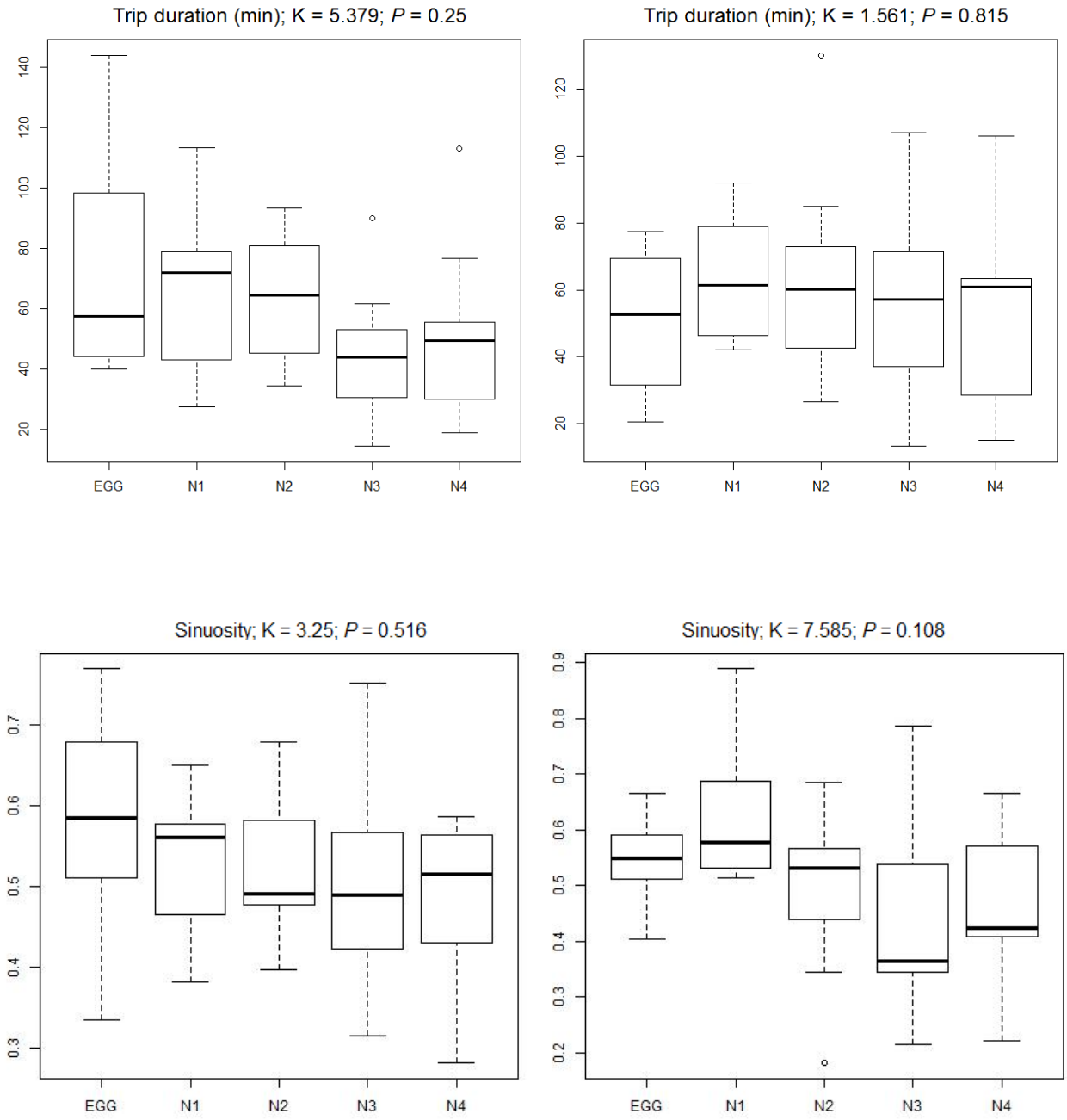

δ15N; K = 4.814; P = 0.306
13.0
12.5
12.0
11.5
EGG
N1
N2
N3
N4


Total distance traveled (km); K = 8.460; P = 0.07
100
80
60
40
20
EGG
N1
N2
N3
N4


Total distance traveled (km); K = 2.368; P = 0.668
70
60
50
40
30
20
10
EGG
N1
N2
N3
N4

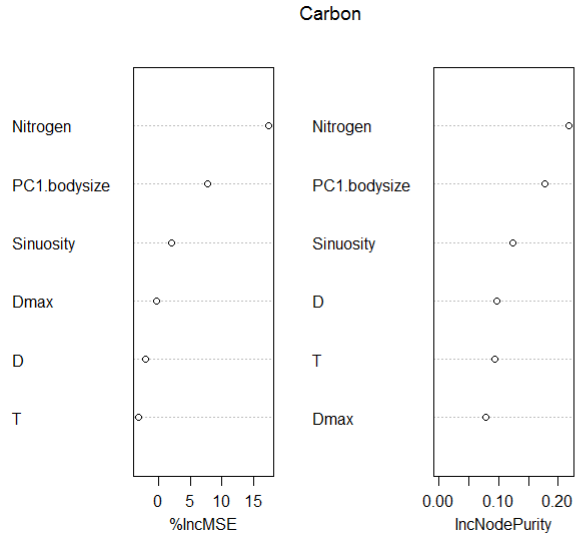

Maximum distance from colony (km); K = 10.144; P = 0.038
15
10
5
EGG
N1
N2
N3
N4

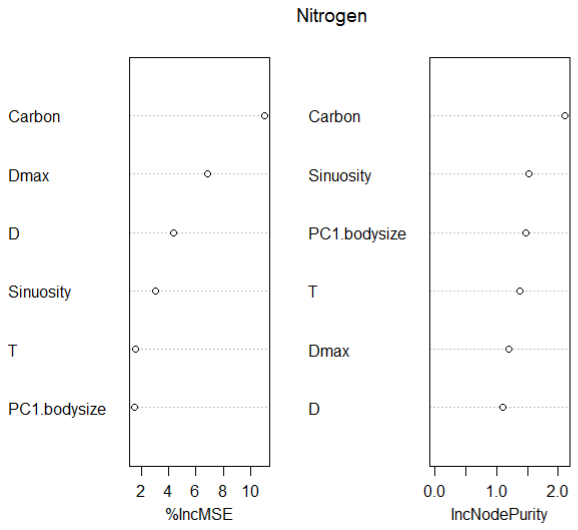

Maximum distance from colony (km); K = 3.360; P = 0.499
20
15
10
5
EGG
N1
N2
N3
N4



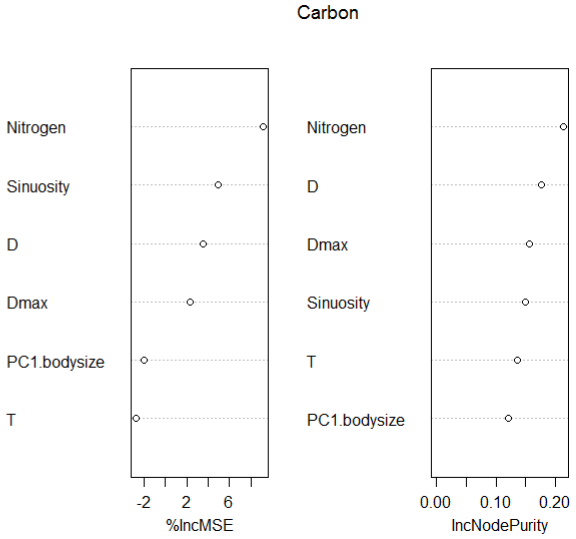
Online Resource 5 Comparisons of diet and foraging behavior parameters between brown boobies *Sula leucogaster* in distinct breeding stages in the Saint Peter and Saint Paul Archipelago, in July 2015. The non-parametric Kruskal-Wallis test (K) was performed to assess significant differences between breeding stages. Chicks were aged using the classification suggested by Simmons (1967): N1 (0–3 weeks); N2 (4–6 weeks); N3 (7–11 weeks); N4 (12 weeks to fledgling)

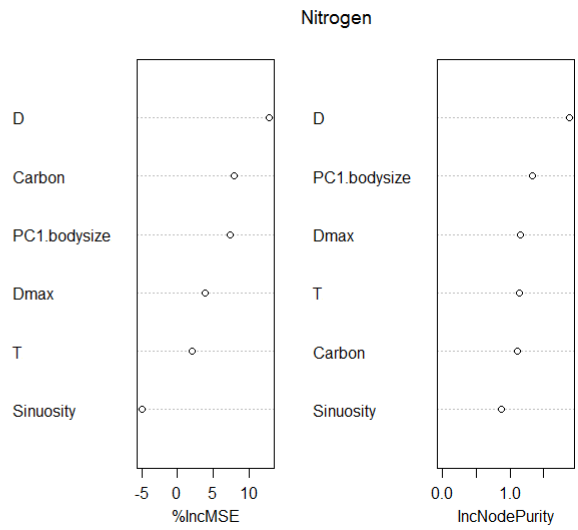
♀

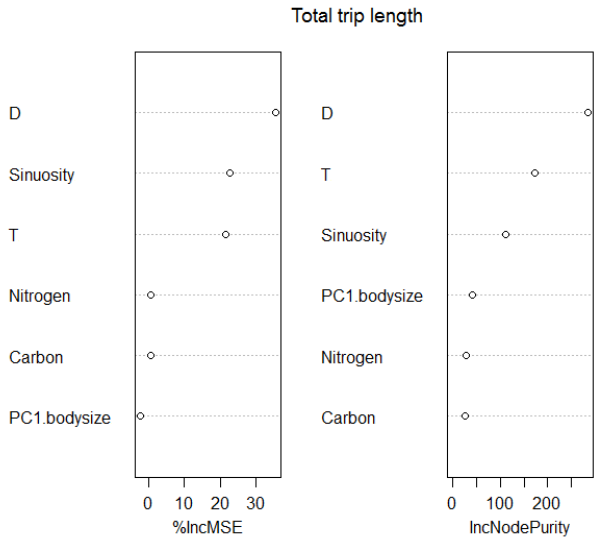

Carbon
Nitrogen
PC1.bodysize
Sinuosity
Dmax
D
T
0 5 10 15
%IncMSE
Nitrogen
PC1.bodysize
Sinuosity
D
T
Dmax
0.00 0.10 0.20
IncNodePurity

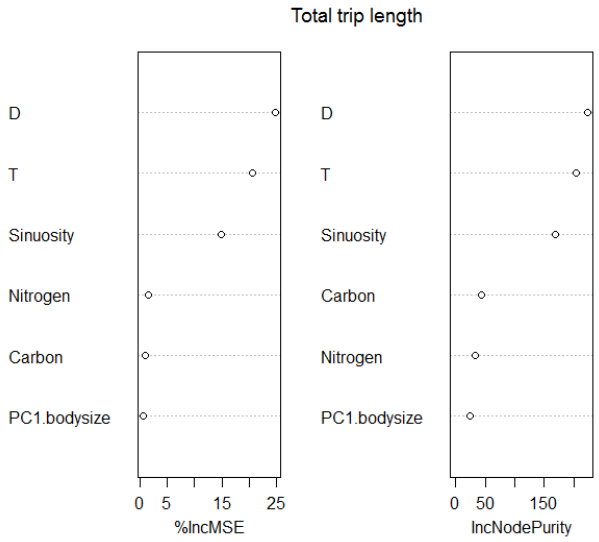

Nitrogen
Carbon
Dmax
D
Sinuosity
T
PC1.bodysize
2 4 6 8 10
%IncMSE
Carbon
Sinuosity
PC1.bodysize
T
Dmax
D
0.0 1.0 2.0
IncNodePurity

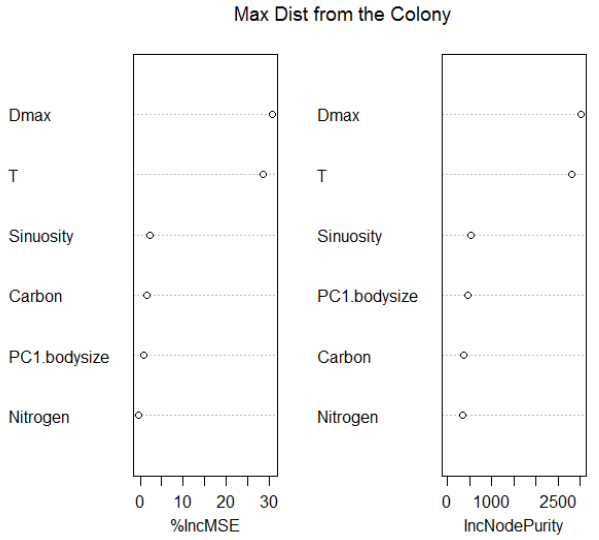
♂

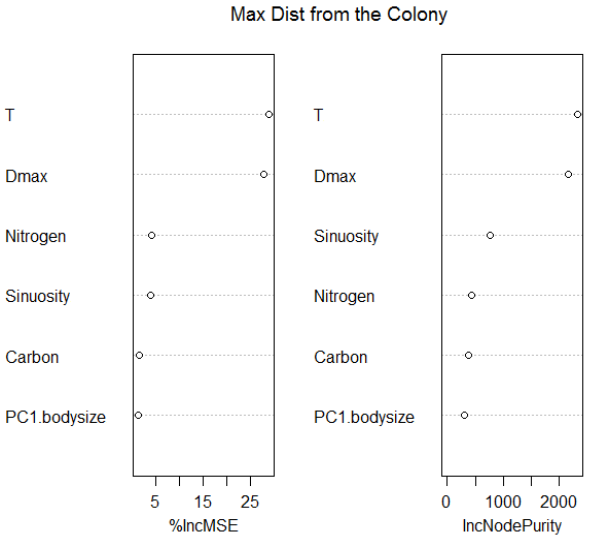

Carbon
Nitrogen
Sinuosity
D
Dmax
PC1.bodysize
T
-2 2 6
%IncMSE
Nitrogen
D
Dmax
Sinuosity
T
PC1.bodysize
0.00 0.10 0.20
IncNodePurity

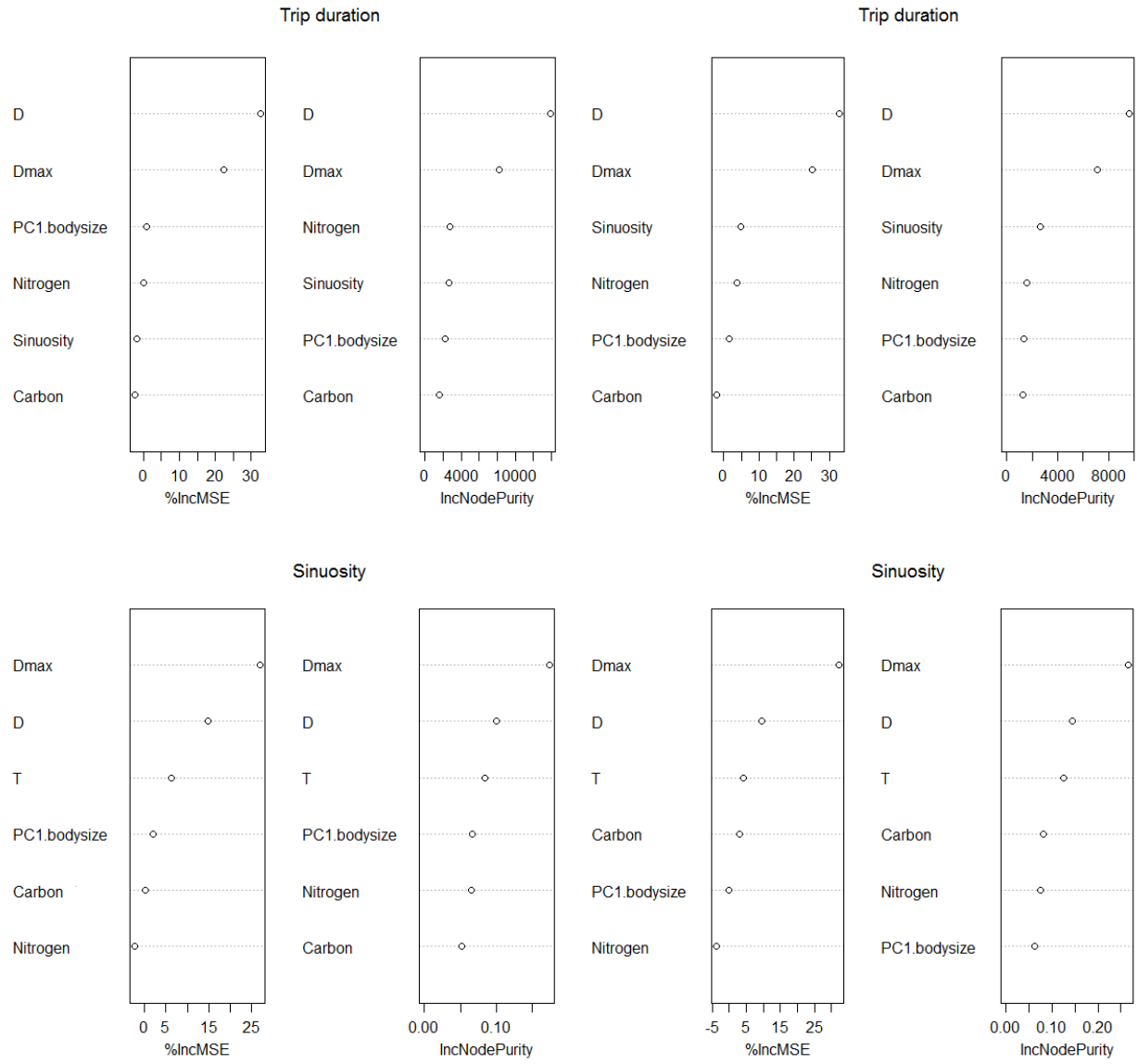

Nitrogen
D
Carbon
PC1.bodysize
Dmax
T
Sinuosity
-5 0 5 10
%IncMSE
D
PC1.bodysize
Dmax
T
Carbon
Sinuosity
0.0 1.0
IncNodePurity


Total trip length
D
Sinuosity
T
Nitrogen
Carbon
PC1.bodysize
0 10 20 30
%IncMSE
D
T
Sinuosity
PC1.bodysize
Nitrogen
Carbon
0 100 200
IncNodePurity


Total trip length
D
T
Sinuosity
Nitrogen
Carbon
PC1.bodysize
0 5 15 25
%IncMSE
D
T
Sinuosity
Carbon
Nitrogen
PC1.bodysize
0 50 150
IncNodePurity


Max Dist from the Colony
Dmax
T
Sinuosity
Carbon
PC1.bodysize
Nitrogen
0 10 20 30
%IncMSE
Dmax
T
Sinuosity
PC1.bodysize
Carbon
Nitrogen
0 1000 2500
IncNodePurity


Max Dist from the Colony
T
Dmax
Nitrogen
Sinuosity
Carbon
PC1.bodysize
5 15 25
%IncMSE
T
Dmax
Sinuosity
Nitrogen
Carbon
PC1.bodysize
0 1000 2000
IncNodePurity



Online Resource 6 Importance of dependent variables in models performed with 1000 regression trees and 20 per tree permutations for out-of-bag data, through the Random Forest algorithm. %IncMSE represent how worse the model performs without each variable, so that very predictive and important variables are characterized by a high decrease in accuracy (variables at the top). IncNodePurity measures how pure the nodes are at the end of the tree. Carbon = $\delta^{13}C$; Nitrogen = $\delta^{15}N$; D = mean total distance ('Total trip length'); Dmax = mean maximum distance from colony ('Max Dist from the Colony'); T = trip duration; Sinuosity = $Dist.2Dmax^{-1}$; PC1.bodysize = body size index composed by culmen length, tarsus length, wing chord, and body mass data converted in a principal component (PC1)