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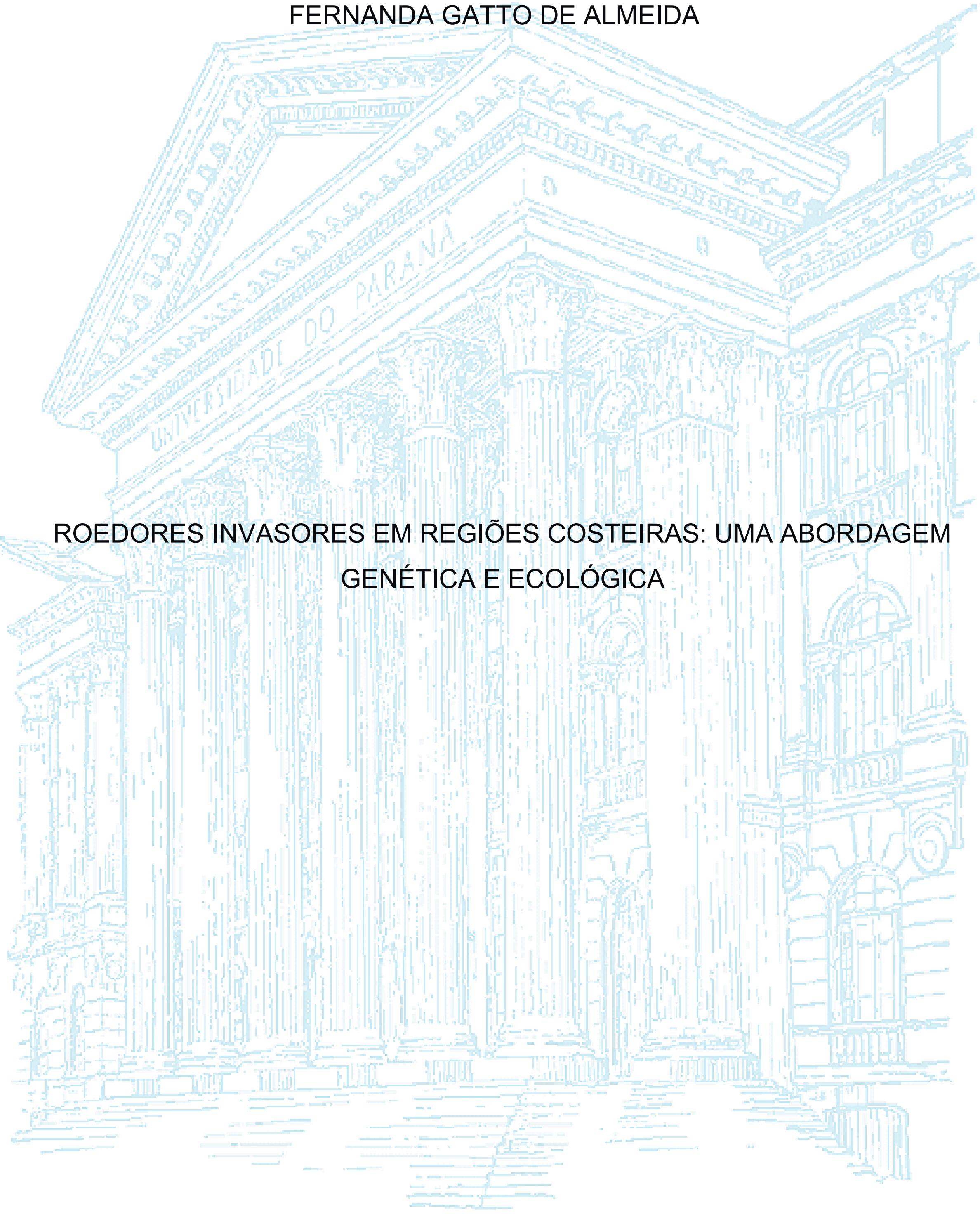
FERNANDA GATTO DE ALMEIDA



ROEDORES INVASORES EM REGIÕES COSTEIRAS: UMA ABORDAGEM GENÉTICA E ECOLÓGICA

CURITIBA
2019

FERNANDA GATTO DE ALMEIDA

ROEDORES INVASORES EM REGIÕES COSTEIRAS: UMA ABORDAGEM GENÉTICA E ECOLÓGICA

Tese apresentada ao Programa de Pós-Graduação em Genética, Setor de Ciências Biológicas da Universidade Federal do Paraná como requisito parcial à obtenção do título de Doutora em Genética.

Orientadora: Prof^{a} Dra. Iris Hass
Coorientadora: Prof^{a} Dra. Liliani Marilia Tiepolo
Coorientador: Prof^{o} Dr. James C. Russell

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em GENÉTICA da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **FERNANDA GATTO DE ALMEIDA** intitulada: **Roedores invasores em regiões costeiras: uma abordagem genética e ecológica**, sob orientação da Profa. Dra. IRIS HASS, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua Aprovação no rito de defesa.

A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 30 de Outubro de 2019.

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“What you do makes a difference, and you have to decide what kind of difference you want to make.”

— Jane Goodal

RESUMO

A invasão biológica por espécies exóticas é considerada uma das maiores ameaças ao bem-estar ecológico do planeta. Ratos e camundongos são os mamíferos invasores mais disseminados pelo mundo. A bioinvasão não é uma área consolidada de pesquisa no Brasil, e até 2013, apenas 16% dos trabalhos produzidos com espécies não nativas tinham mamíferos como objeto de estudo. Esta tese se propõe a dar continuidade aos primeiros estudos com roedores invasores no Brasil, e preencher algumas lacunas de informações através de uma abordagem ecológica e genética. No capítulo I foi realizada a modelagem de ocupação de duas espécies de roedores exóticos detectadas no Município de Paranaguá, *Rattus norvegicus* e *Mus musculus.* Neste trabalho, foi detectada uma relação negativa entre a probabilidade de ocupação dessas espécies e a porcentagem de cobertura florestal em um raio de 1000m. Esta análise permitiu propor que as fitofisionomias florestais do bioma Mata Atlântica servem como uma barreira de dispersão para as espécies estudadas. No capítulo II, foram utilizados marcadores de microssatélites para avaliar o fluxo gênico do roedor *Rattus norvegicus* ao longo de uma das principais vias de acesso ao porto de Paranaguá. Constatamos uma significativa frequência de dispersão mediada por humanos que aproxima geneticamente as duas populações mais afastadas geograficamente. E finalmente no capítulo III foram utilizadas amostras de *Rattus rattus* provenientes do arquipélago de Fernando de Noronha para se avaliar o fluxo gênico entre as populações da cadeia de ilhas a nordeste pertencentes ao arquipélago. Através do uso de microssatélites e sequenciamento da região mitochondrial d-loop os resultados indicaram ausência de fluxo gênico entre as ilhas, o que faz da ilha Rata a melhor candidata ao controle e a erradicação.

Palavras Chave: *Rattus*, *Mus*, modelagem de ocupação, microssatélites, d-loop

ABSTRACT

Biological invasion by exotic species is considered one of the greatest threats to the planet's ecological welfare. Rats and mice are the most widespread invasive mammals in the world. Bioinvasion is not a consolidated research area in Brazil, and until 2013, only 16% of the papers published with non-native species had mammals as their object of study. This thesis aims to continue the first studies with invasive rodents in Brazil, and fill some information gaps through an ecological and genetic approach. In chapter I, the occupancy modeling of two exotic rodent species detected in Paranaguá, *Rattus norvegicus* and *Mus musculus*, was performed. In this work, a negative relationship was detected between the probability of occupation of these species and the percentage of forest cover within a 1000m radius. This analysis allowed us to propose that the forest phytophysiognomies of the Atlantic Forest biome serve as a dispersal barrier for the studied species. In Chapter II, microsatellite markers were used to evaluate the gene flow of the *Rattus norvegicus* rodent along one of the main roads used to access to the port of Paranaguá. We found a significant frequency of human-mediated dispersion that genetically approximates the two geographically distant populations. Finally, in Chapter III, samples of *Rattus rattus* from the Fernando de Noronha archipelago were used to evaluate the gene flow among the populations of the northeastern island chain belonging to the archipelago. Using microsatellites and sequences of the mitochondrial d-loop region, the results indicated the absence of gene flow between the islands, which makes Rata Island a great candidate for control and eradication.

Key words: *Rattus*, *Mus*, occupancy modeling, microsatellites, d-loop

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1. INTRODUÇÃO

A invasão biológica por espécies exóticas é hoje considerada uma das maiores ameaças ao bem estar ecológico do planeta (Matthews e Brand, 2005), e pode ser definida como: o fenômeno onde uma espécie adquire uma vantagem competitiva após o desaparecimento de um obstáculo natural para sua proliferação, o que lhe permite se espalhar rapidamente e conquistar novas áreas do ecossistema receptor, na qual se torna uma espécie dominante (Valéry et al., 2008).

A chegada de espécies exóticas a novas áreas se dá geralmente por transporte acidental em meio à água de lastro de navios e embarcações (no caso de espécies aquáticas) ou em carregamentos de navios, aviões, caminhões e demais meios de transporte utilizados pelo homem (Sanu e Newport, 2010), no caso de espécies terrestres. Dentro desta última possibilidade, estão os ratos, considerados os mamíferos invasores mais disseminados pelo mundo (Drake e Lodge, 2004; Russell et al., 2008).

Quatro espécies de roedores são comumente consideradas problemáticas invasoras e têm larga distribuição global: o rato preto (*Rattus rattus* Linnaeus, 1758), rato marrom ou norueguês (*R. novegicus* Berkenhout, 1769), rato do Pacífico ou da Polinésia (*R. exulans* Peale, 1848), o qual possui um distribuição mais restrita e não ocorre no Brasil, e o camundongo doméstico (*Mus musculus* Linnaeus, 1758) (Carleton e Musser, 2005; Harris 2009). A espécie *R. rattus* é considerada originária da Índia e se dispersou no mundo todo através de navios, razão pela qual o roedor também é conhecido como rato de navio. O *R. norvergicus*, o maior deles, é originário da China e disseminou-se na Europa no início do século XVIII, a partir de onde foi transportado em navios pelo resto do mundo. Este roedor é ótimo nadador podendo nadar por até 1 km (Matthews e Brand, 2005; Russell et al., 2008). A espécie *M. musculus* tem sua origem no norte do subcontinente Indiano de onde se dispersou para o resto do mundo devido à sua associação comensal com humanos. Apesar de comumente ocupar construções, podem viver em diversos ambientes, de campos a florestas (Boursot et al. 1996; Atkinson e Atkison, 2000; Cucchi e Vigne, 2007).

Entre os impactos negativos causados por esses roedores podemos citar: (1) prejuízo econômico, em 2000, Pimentel et al. estimaram prejuízo de 19 bilhões de dólares por ano, nos Estados Unidos, causado por roedores invasores que consomem ou destroem grãos e outros materiais estocados. (2) Risco de transmissão de doenças, as quais incluem febre por mordedura (Sodoku), tifo murinho, coriomeningite linfocitária, salmonelose, hantaviroses, leptospirose entre outras. No Brasil estima-se que ocorram anualmente mais de 3000 casos de leptospirose humana, com mortalidade de 12% (FUNASA, 2002 e Kimura, 2002). (3) Impacto direto na fauna local, como a predação de aves marinhas resultando em declínio populacional e algumas vezes extinção da população local fenômeno já foi registrado globalmente (Jones et al., 2008).

Um levantamento sistemático feito por Lowry et al. (2013), que até 2011 existia uma escassez de estudos de campo sobre invasão biológica nos trópicos (Figura 1). Especificamente na América do Sul, onde os trabalhos existentes se concentraram principalmente no Chile, e alguns poucos na Argentina, enquanto no Brasil o baixo número de trabalhos era evidente. Por outro lado, as regiões da América do Norte, Europa Ocidental, Austrália Oriental, Nova Zelândia e Hawaii, foram identificadas com maior abundância desses estudos. Somente nos últimos anos estudos sobre bioinvasão começaram a se despontar no Brasil peixes (e.g. Zenni, 2014; 2015; Zenni et al., 2016; Frehse et al., 2016; Da Rosa et al., 2017).

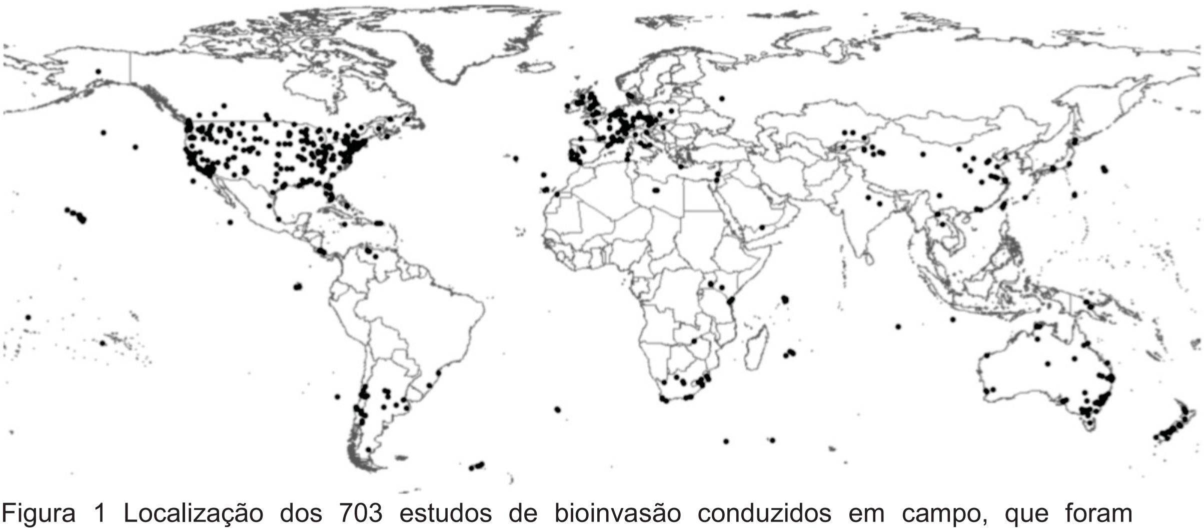

Figura 1 Localização dos 703 estudos de bioinvasão conduzidos em campo, que foram identificados no trabalho de Lowry et al. (2013). Retirado de Lowry et al. (2013).

Além disso, no Brasil, os estudos com espécies não-nativas começaram a aparecer quase uma década após o resto do mundo (por volta de 1998) e apenas 16% dos trabalhos produzidos com espécies não-nativas até 2013 tinham mamíferos como objeto de estudo (Frehse et al., 2016).

Diante destes dados fica claro que no Brasil, estamos deficitários nas pesquisas sobre invasão biológica e mais ainda naquelas que dizem respeito a mamíferos, como ratos e camundongos. A despeito da diferença do número de pesquisas sobre invasão biológicas no Brasil em relação a outros países, já é possível vislumbrar algumas dimensões do problema de roedores exóticos no país. São pioneiros os trabalhos de Kajdacsi et al. (2013) e Richardson et al. (2017) onde os autores avaliaram a estruturação genética e propuseram unidades de erradicação para a espécie *R. norvegicus* em uma favela da cidade de Salvador, Bahia. Para a mesma localidade, Panti-may et al. (2016) descreveram padrões sazonais de abundância, reprodução e morfometria em um estudo de amostragem massiva que durou dois anos.

Além desses estudos, espécies exóticas de roedores aparecem como registros esporádicos em estudos de levantamento em mata nativa realizados no Brasil, como no caso do Parque Estadual do Rio Doce em Minas Gerais (Stallings et al., 1991), de uma floresta com araucárias em Santa Catarina (Cherem e Perez, 1996) e de uma mata de Restinga no Rio Grande do Sul (Quintela et al., 2012), indicando assim que os roedores nativos estão em contato direto com os exóticos, possivelmente sendo negativamente impactados (Harris, 2009).

A presente tese visa dar continuidade aos estudos na área de bioinvasão por roedores exóticos, inciados no Brasil por Kajdacsi et al. (2013), Panti-may et al. (2016) e Richardson et al. (2017). No presente estudo trabalhamos de maneira multidisciplinar visando esclarecer diferentes aspectos de tal problemática no canário nacional, como: (I) padrão de ocupação preferencial no ambiente urbano e de floresta, (II) dinâmica de dispersão em ambiente urbano e (III) viabilidade de um programa de erradicação em ambiente insular.

Os dois primeiros capítulos são resultado de nove campanhas de campo realizadas no período de Janeiro de 2017 a Julho de 2018, na cidade portuária de Paranaguá/PR (25°31′S, 48°30′W). Ao todo foram amostrados 18 pontos de coleta/amostragem que compreenderam ambientes urbanos, de borda e de interior de floresta, totalizando esforço amostral de 3.512 armadilhas.noite e 702 baldes.noite. Para o capítulo II também foram incluidas amostras provenientes de busca ativa por carcaças no ambiente urbano, além de material cedido pela adminstração dos portos de Paranaguá e Antonina (APPA), através de parceria firmada via ofício nº 657/2017 – APPA/EP de 25 de Julho de 2017.

O capítulo III é resultado de parceria firmada com o laboratório do profº Dr. James C. Russel, da University of Auckland. As amostras desse capítulo foram coletadas no archipélago de Fernando de Noronha/PE (3º50’S, 32º26’W), pela equipe de pesquisadores do Instituto Brasileiro para Medicina da Conservação (TRÍADE) no período de 2015 a 2018. Os procedimentos laboratóriais foram desenvolvidos na University of Auckland durante período de intercâmbio que ocorreu no período de Outubro de 2018 a Março de 2019.

2. OBJETIVOS

2.1 OBJETIVO GERAL

- Mensurar parâmetros genéticos populacionais e ecológicos de três espécies de roedores exóticos dimensionando o cenário atual de bioinvasões destes organismos em áreas costeiras no Brasil.

2.2 OBJETIVOS ESPECÍFICOS

- Reconhecer quais as espécies de roedores invasores presentes em Paranaguá;
- Entender como ratos e camundongos invasores se relacionam com a paisagem da Mata Atlântica;
- Descrever padrões de ocupação de roedores invasores na cidade portuária de Paranaguá;
- Gerar estimativas de densidade para as espécies de roedores invasores detectadas;
- Determinar intensidade de dispersão e estruturação genética dentro da cidade portuária de Paranaguá;
- Determinar unidades de erradiçãoerradicação no arquipelágo de Fernando de Noronha;
- Avaliar a factibilidade da proposta de erradicação já existente para o arquipélago.

3. REVISÃO BIBLIOGRÁFICA

3.1 Roedores Invasores

Roedores formam a ordem de mamíferos mais rica em espécies do mundo, com 2277 espécies descritas até 2005 (Carleton e Musser, 2005) e muitas outras descritas desde então (e.g. Tavares et al., 2011; Percequillo et al., 2011; Quintela et al., 2014; Christoff et al., 2016). Estes animais têm distribuição cosmopolita e possuem representantes nativos em todas as regiões exceto Antártica, Nova Zelândia e algumas ilhas oceânicas (Feldhamer et al., 2007).

Embora a maioria das espécies não apresente impacto negativo sobre o homem, 147 espécies de roedores são consideradas 'pestes' no mundo moderno, dentre as quais se destacam *Mus musculus* (Figura 2A), *Rattus rattus* (Figura 2B) e *Rattus norvegicus* (Figura 2C) como as mais importantes (Capizzi et al., 2014). Tais espécies apresentam comportamento comensal e se espalharam pelo mundo através do transporte acidental mediado por humanos, se tornando mais abundantes nos ambientes urbanos do que em seus habitat naturais (Long, 2003). A seguir serão apresentadas algumas das características de tais espécies:

3.1.1 Mus musculus

O roedor *Mus musculus*, conhecido como camundongo de casa é um muróideo pertencente à família Muridae. Seu gênero compreende 38 espécies que possuem larga distribuição pelo velho mundo. Porém, apenas *M. musculus* é considerada uma espécie invasora (Suzuki et al., 2004; Carleton e Musser, 2005). Essa espécie se subdivide ainda em ao menos três subspécies (*M. m. castaneus, M. m. musculus* e *M. m. domesticus*), que se acredita terem origem na região que hoje compreende Irã, Afeganistão, Pasquistão e norte da India (Suzuki et al., 2013).

Quando em ambiente urbano, geralmente mantém o comportamento comensal, ocupando locais cobertos e com estrutura complexa, como habitações humanas, áreas de resíduos e outros habitats antropogênicos

(Jensen et al., 2003; Pocock et al., 2005). Porém, em alguns cenários podem formar populações afastadas de construções humanas como em plantações, ou ambientes naturais como ilhas (Jones et al., 2003; Pocock et al., 2004).

Figura 2 Espécies de roedores invasores abordadas na presente tese. *Mus musculus* (A); *Rattus rattus* (B); *Rattus norvegicus* (C). Fonte: a autora.

Quando introduzidos em ambiente de ilha, camundongos se alimentam de plantas, invertebrados, ovos de aves e até mesmo filhotes de aves causando impactos devastadores que normalmente somente são associados a ratos maiores (Angel et al., 2009; Shiels et al., 2013). Acredita-se que quando *Mus musculus* ocupa uma área também ocupada por outras espécies de roedores, sua densidade é suprimida até que o competidor seja eradicado do ambiente (Witmer et al., 2007; Caut et al., 2007). Por sua vez, sua erradicação tem se mostrado menos eficiente do que a de espécies de *Rattus*, embora não exista um consenso sobre o motivo de tal falha (MacKay et al., 2007).

3.1.2 Rattus rattus

O gênero *Rattus* compreende 66 espécies de roedores alocados dentro da família Muridae. Dentre essas espécies, três se destacam por seu comportamento comensal e invasor de habitats: *Rattus exulans* (que não será discutido aqui), *Rattus rattus* e *Rattus norvegicus* (Carleton e Musser, 2005).

A espécie *Rattus rattus*, também conhecida como rato preto, ou rato de telhado possui sistemática controversa e estudos recentes sugerem que o "complexo *Rattus rattus*" é polifilético incluindo, pelo menos, quatros espécies (Aplin et al., 2011). A linhagem I, reconhecida por Aplin et al. (2011), se originou no sul da India e possui a distribuição mais abrangente fora do continente asiático, com representantes na Europa, Américas, Africa, Madagascar, Australia e várias ilhas do Pacífico. Além dessa, somente a linhagem II foi introduzida no continente Americano, porém é encontrada apenas na parte ocidental dos Estados Unidos.

Essa espécie pode ocupar uma grande variedade de ambientes que incluem: ilhas, florestas, e cidades (Harper et al., 2015; Mangombi et al., 2016; Walker et al., 2019), porém sua abundância nestes ambientes parece ser fortemente determinada pela presença de competição no ambiente (King et al., 2011). De acordo com King et al. (2011), até o século 18, o rato preto era o único rato comensal na Grã-Bretanha e após a chegada do *R. norvegicus*, foi deslocado do solo do ambiente urbano para ambientes verticais como sótãos ou construções e florestas.

É a espécie que causa maior número conhecido de impactos negativos sobre a fauna de ilhas (Harper e Bunbury, 2015). Sua habilidade de escalar árvores parece favorecer sua dominância em ambientes florestais de ilha, muito embora a presença de vegetação não seja pré-requisito para sua sobrevivência fora do ambiente urbano (Shiels et al., 2014; Harper e Bunbury, 2015). Regiões tropicais também parecem estar relacionadas ao maior sucesso de ocupação por essa espécie, uma vez que baixas temperaturas têm se mostrado ligadas à diminuição do período reprodutivo e da densidade populacional (Feng e Himsworth, 2014; Harper e Bunbury, 2015; Walker et al., 2019).

3.1.3 Rattus norvegicus

A espécie *Rattus norvegicus*, também conhecida como rato-marrom ou rato norueguês, se originou no sul da China há aproximadamente 1.3 milhão de anos (Song et al., 2014) e começou sua expansão tardiamente se comparado às outras duas espécies, por volta de 1500 (Puckett et al., 2016). Dentre as três espécies aqui tratadas é a maior podendo alcançar até 900g (Long, 2003).

Ao contrário do rato preto, preferem foragear no solo, e em ambiente urbano, acessam pontenciais abrigos utilizando o sistema de esgoto ou mesmo buracos nas fundações de prédios. Embora possam coabitar ocupações humanas, locais abandonados parecem ter preferência (Fosterat et al. 2011; Feng e Himsworth, 2014). Ratos noruegueses também podem criar seus próprios abrigos cavando buracos no chão, inclusive já foi demonstrado que esta espécie é menos abundante em regiões urbanas que possuem pouco solo natural disponível (Traweger et al., 2006).

Quando presente em ilhas, *R. norvegicus* frequentemente se encontra restrito a áreas de agricultura ou com assentamento humano (Harper e Bunbury, 2015). Pouco se sabe sobre seu impacto na fauna nativa de ilhas, porém foi registrado aumento nas populações de duas espécies de aves marinhas assim como aumento na cobertura vegetal na Ilha Tromelin, após a erradicação da população de rato norueguês (Corre et al., 2015).

3.2 Modelagem de Ocupação

Um desafio comum na ecologia é o paradoxo da escala: fenômenos como perda de diversidade e aquecimento global acontecem em grandes escalas de tempo e espaço, porém a coleta de dados é tipicamente feita em um curto período de tempo e em uma área limitada (Levin, 1992; Miller et al., 2004; Urban, 2005). Devido às limitações financeiras, logísticas e técnicas que investigações mais amplas encontram, entender padrões que acontecem em grande escala depende principalmente da capacidade de se fazer extrapolações confiáveis usando os dados coletados em menor escala (Miller et al., 2004).

Extrapolações podem ser feitas a partir da criação de modelos, que nada mais são do que formas de descrever um padrão observado dentro de um sistema. Um modelo matemático usa equações matemáticas para traduzir tais padrões e assim prever como o sistema muda em resposta às mudanças (extrapolar os dados) (MacKenzie et al., 2018).

Em termos práticos: para a investigação da distribuição de uma espécie dentro de um bioma, seria impraticável investigar toda a sua extensão. Ao invés disso, o pesquisador pode reunir informações sobre as ocorrências conhecidas e condições ecológicas encontradas em tais localidades (e.g. temperatura, tipo de cobertura vegetacional). Utilizando de modelagem, ao aliar estas infromações será possivel mapear a área geográfica que possui a mesma combinação de condições e então prever a distribuição da espécie alvo (Ricklefs, 2010). Sua aplicação vai além, a modelagem é usada nos mais diversos campos de pesquisa e pode ser usada para prever desde áreas prioritárias para a conservação de espécies (Rodríguez-Soto et al., 2011) até distúrbios naturais como secas, ventanias, incêndios (Seidl et al., 2011).

Especificamente a modelagem de ocupação (i.e. abordagem que avalia a probabilidade de ocupação de uma espécie em um determinado local) descrita por Mackenzie et al. (2002) têm sido utilizada em estudos de conservação devido à sua versatilidade (Thorn et al., 2011). Esse tipo de análise requer dados sobre os eventos de detecção/não detecção da espécie alvo, leva em consideração o fato de que as espécies não são detectadas 100% das vezes (probabilidade de detecção) e pode ser usado para fazer inferências a respeito de grandes áreas através da amostragem de pequenas parcelas (Mackenzie et al., 2002).

Esse método de modelagem utiliza uma abordagem parecida com aquela utilizada no método de marcação e recaptura (Otis et al., 1978), porém com um parâmetro adicional (Ψ - Psi) que representa a probabilidade de ocupação da espécie. A probalidade de ocupação é calculada para cada um dos sítios amostrais, independentemente, através do registro de todos os eventos de deteção e não detecção da espécie naquele sítio. Um evento de detecção será incorporado à modelagem através da expressão Ψ × P, onde Ψ significa probalidade de ocupação e P a probabilidade de detecção. Os eventos de não detecção também são incorporados levando-se em conta a possilidade da espécie estar presente e não ter sido detectada (Ψ × [1–P]), e a possibilidade da espécie não estar presente (1–Ψ) (Mackenzie et al., 2018).

Nos últimos anos, essa metodologia tem se tornado popular principalmente com estudos de mamíferos de médio e grande porte que utilzam camera trap em sua amostragem (e.g. Duquette et al., 2017; Ferreguetti et al., 2017; Ferreguetti et al., 2018; Rodrigues e Chiarello, 2018). Estudos com pequenos mamíferos que usam esta abordagem são mais raros, mas começaram a aparecer para elucidar algumas questões ecológicas. Luza et al. (2018) avaliaram a probabilidade de ocupação de três roedores nativos em áreas sujeitas a diferentes intensidades de pastejo nos campos sulinos, e duas delas apresentaram maior probabilidade de ocupação em ambientes não pastados. Mais recentemente, Serafini et al. (2019) relacionaram a probabilidade de ocupação de uma assembleia de pequenos mamíferos à complexidade do ambiente (índice de Shannon).

Embora muita informação já tenha sido reunida a respeito da ecologia de roedores invasores já ao longo dos anos (Feng e Himsworth, 2014), é sabido que esses roedores podem apresentar diferentes comportamentos em decorrência das complexas interações que estabelecem nos ambientes que colonizam (King et al., 2011). Dessa forma, para entender como esses animais se relacionam com os biomas unicos encontrados no Brasil, como a Mata Atlântica, se faz necessário a aplicação deste tipo de análise que se mostra promissora no sentido de gerar uma visão ampla para uma área onde se tem pouca informação.

3.3 Microssatélites e Roedores Invasores

Microssatélites também conhecidos como sequências de repetição simples (SSR), sequências de repetição em tandem (SRT) ou polimorfismos de comprimento de sequência simples (SSLP) são sequêcias de DNA formadas por unidades de repetição de tamanho menor que 5 pares de base, que se posicionam em tandem (uma após a outra), ocorrendo em abundância pelo genoma eucarioto (Bruford e Wayne, 1993). Estas sequências apresentam altos níveis de polimofismo em decorrência da variação no número de repetições dentro de cada *locus*, além disso, sofrem baixa pressão seletiva e possuem relação de codominância entre alelos (alelos distintos são identificáveis nos heterozigotos) o que os torna ótimos marcadores genéticos para estudos de população (Jarne e Lagoda, 1996; Sunnucks, 2000).

Desde o desenvolvimento da técnica de PCR (Polymerase Chain Reaction) no fim da década de 80, microssatélilteS têm sido usados largamente em estudos da área de patologia, filogenética e genética da conservação (Jarne e Lagoda, 1996; Vieira et al., 2016). A utilização destes marcadores em estudos com roedores invasores começou logo cedo, e já em 1990 foram caracterizados os primeiros *primers* para estudos com *Mus musculus* (Love et al., 1990).

Pelo fato de ratos e camundongos serem modelos de estudo foi necessário o desenvolvimento de um maior número de primers que foram publicados ao longo da década de 90. Em 1991 Hearne et al. caracterizaram 74 novos marcadores para *Mus musculus*, e em 1994, Dietrich et al. publicaram o mapa genômico desta espécie descrevendo 4.006 polimorfismos de comprimento de sequência simples (SSLP) (ou microssatélites. E em 1995, Jacob et al. descreveu o mapa de ligação do genoma de *Rattus norvegicus*, o “rato de laboratório”, disponibilizando 432 microssatélites.

Em 1997 Kloting et al. utilizaram pela primeira vez os primers de microssatelite em ratos selvagens com a intenção de avaliar a diversidade alélica de animais selvagens em relação a linhagens de laboratório. Até então tais marcadores só tinham sido usados em linhagens de laboratório para mapear genes de interesse na área médica (e.g. Pravenec et al., 1995; Vyse e Todd, 1996).

A partir da metade da década de 90, o número de estudos com bioinvasão começaram a aumentar (Lowry et al., 2013) e no começo dos anos 2000 foi publicado o primeiro trabalho utilizando microssatélites para avaliar a viabilidade de erradicação de *R. norvegicus* em um complexo de ilhas no oeste europeu (Calmet et al., 2001). Desde então, estes marcadores têm se mostrado poderodas ferramentas no auxílio do planejamento de erradicações de populações de ratos invasores, uma vez que ao traçar o perfil genético de populações alvo e suas adjacentes é possível identificar o risco de reinvasão naquela unidade e definir “unidades de erradicação” (Fewster et al., 2011).

Quando microssatélites começaram a ser utilizados em prol da conservação as conclusões se baseavam exclusivamente em índices estatísticos como estatística-F desenvolvida por Wright (1951), frequência de alelos e alelos privados (e.g. Calmet et al., 2001; Yu et al., 2002; Robertson e Gemmell, 2004). Embora esses índices sejam utilizados até hoje, a análise dos dados de microssatélite foi refinada ao longo dos anos, novas abordagens estatísticas e gráficas foram desenvolvidas (e.g. Pritchard et al., 2000; Evanno et al., 2005; McMillan e Fewster, 2017) e hoje são utilizadas em conjunto para melhor entender a dinâmica das populações em estudos com roedores.

Hoje, microssatélites são tidos como poderosas ferramentas, que auxiliam não somente na definição de unidades de erradicação, mas também em outras questões relacionadas a roedores invasores e em diversas escalas, como por exemplo: Richardson et al. (2017) identificaram que a movimentação de *R. norvegicus* é fortemente impedida pela presença de uma rodovia de tráfego pesado, em uma favela na cidade de Salvador. Brouat et al. (2014)

usaram esses marcadores para inferir caminhos históricos de invasão de *R. rattus* em Madagascar, chegando à conclusão de que dois eventos independentes de introdução, e não um como se acreditava, deram origem àquela população. E Russell et al. (2010) se valeram de métodos de "genetic assigment" para determinar se o fracasso de um programa de erradicação realizado na Ilha Pearl (Nova Zelândia) teria sido causado por sobreviventes da antiga população ou novos invasores provenientes de populações adjacentes.

3.4 Continente versus Ilhas

Quando se trata do manejo de espécies invasoras existem três possíveis objetivos: (1) erradicação (i.e. completa remoção da espécie na área, sem perspectiva imediata de recolonização a partir de área adjacentes), (2) remoção completa da área, porém com a necessidade de vigilância contínua para manter a área livre de recolonização (pode incluir uma zona de amortecimento ou cerca) ou (3) controle dentro de uma área para reduzir a abundândia e o risco associado à espécie (Robertson et al., 2017).

A erradicação é o objetivo máximo quando se trata do manejo desses organismos, porém para que um programa de erradicação obtenha sucesso deve-se garantir que (1) a taxa de remoção seja maior do que a taxa de crescimento da população, (2) novas invasões sejam prevenidas e (3) todos os indivíduos em estado reprodutivo ou com potencial para alcançá-lo sejam removidos (Bomford e O'Brien, 1995).

Embora erradicações no continente sejam possíveis, elas são mais raras e demandam maior esforço e investimento, já que nesse ambiente as unidades de erradicação não são confinadas como em ilhas e portanto requerem esforço contínuo para manter o isolamento (Connolly et al., 2009; King et al., 2011b; Robertson et al., 2017). Em ilhas, a menor escala facilita a remoção dos animais e o potencial isolamento possibita a implementação de medidas de biosegurança mais eficientes que podem interceptar os novos invasores antes dos mesmos estabelecerem novas populações (Russell et al., 2008; Robertson et al., 2019).

Obviamente, outros fatores além da escala e isolamento influenciam a taxa de sucesso de programas de erradicação (e.g. presença humana, latitude) (Oppel et al., 2011; Holmes et al., 2015), porém no que diz respeito a roedores das espécies *R. rattus, R. norvegicus* e *Mus musculus*, a erradicação em áreas continentais não é usualmente tida como objetivo, pois a rápida recolonização é provável (King et al., 2011b). Como exceção existe o caso de criação de santuários cercados que são ‘pest-free’ (Miskelly, 2018).

Desta forma, estudos em áreas continentais ou grandes ilhas geralmente focam em entender as dinâmicas e relações desses animais tendo como objetivo final o refinamento do controle populacional e não a erradicação ou remoção (e.g. Himsworth et al., 2013; Mangombi et al., 2016). Por outro lado, estudos direcionados a ambientes insulares têm em sua grande maioria, o objetivo final de erradicação da área (Russell et al., 2010; Fewster et al., 2011; Amos et al., 2016). Porém, devemos ressaltar que mesmo que em 2015 o Database of Invasive Species Eradications (http://diise.islandconservation .org) havia registrado 580 ilhas que obtiveram sucesso na erradicação de ratos (Russell e Holmes, 2015), falhas nestes programas ainda são comuns mesmos em ambientes isolados como ilhas (Kappes et al., 2019).

A costa brasileira possui cinco conjuntos de ilhas oceânicas ao longa da sua costa: Arquipélago de Fernando de Noronha, Ilha da trindade, Arquipélago Martim Vaz, Atol das Rocas e Arquipélago de São Pedro e São Paulo (Mohr, 2009). Dentre estes, apenas Fernando de Noronha deu inicio à investigação a respeito de manejo de roedores invasores (e.g. Russell et al., 2018). E na presente tese, daremos um passo adiante nessa investigação.

4. CAPÍTULO I. ATLANTIC FOREST AS A BARRIER TO INVASIVE RODENTS: A STUDY IN A PORT CITY

(submetido à revista Hystrix)

Atlantic Forest as a barrier to invasive rodents: a study in a port city

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Abstract

Invasive rats and mice arrive in new land through accidental transport aboard ocean-going vessels, what turns seaports into entry doors to new colonizers and immigrants. We were able to model the spatial distribution and abundance of two invasive rodent species using, for the first time, sampling locations in a port city, in Brazil, whose original forest cover is the Atlantic Rain Forest. Occupancy and abundance estimates were assessed using live traps and pitfalls during 9 field campaigns (4.214 traps.night) and the sampling sites were selected along the city according to their distance from the harbour towards the forest. The abundance and occupancy of both species were negatively affected by the percentage of forest cover within radius of 1000m. For *Mus musculus*, occupancy was also affected by the abundance of human edifications within radius of 100m and by the distance until the closest forest edge. *Rattus norvegicus* showed a positive relationship with the proximity to the roads used by trucks to access the port area in Paranaguá city. We conclude that in spite of the harbour be the entrance door to invasive rodents, in Paranaguá, both species analysed did not show strong association with the vessels dock. Besides, we presented elements that are associated with rats and mice occupation providing information so local authorities can start an invasive species management program in Paranaguá municipality. Finally, Atlantic Forest stands as a natural barrier to rats and mice invasion once it is associated with their low abundance and occupancy probability.

Keywords: *Exotic Species; Occupancy; Abundance; Habitat Selection; Management; Urban Species*

Introduction

Rats and mice are among the worst invasive mammals in the world (Lowe et al. 2000). Specifically the Norway rat (*Rattus norvegicus*), Ship Rat (*Rattus rattus*) and House mice (*Mus musculus*) have colonized all continents (except Antarctica) through accidental transport aboard ocean-going vessels (Long 2003, Russell et al. 2008, 2007).

A global fleet of over 50,000 vessels carries more than 10.7 billion tons by sea every year (UNCTAD 2018) and once seaports are major entry points for international trades they are also key points of entry for invasive rats (Hulme 2009, Russell et al. 2008). Prevention measures, such as inspections at ports, to reduce the risk of introduction are the first stage for target settlings, if this fails and a species is released into a new region, the next step is to control establishment and spread, which is dependent on several physical and biological characteristics of the region (Gren 2008). Knowing which characteristics of the new environment determine occupation of invasive rodents can help to improve control measures in port cities and minimize its spreading environment. Although, across the world, several studies have been conducted intending to understand patterns of distribution of rats and mice (Lehtonen et al. 2001, Traweger et al. 2006, Walker et al. 2019) it has been shown that these invaders can present different answers in different contexts (King et al. 2011) and these animals never had their occupancy evaluated in a Brazilian environment.

Brazil is a megadiverse country that holds proximally 9.5% of world's species (Lewinsohn and Prado 2005)and invasive rodents present a threat for a lot of species around the world including plants, invertebrates, birds and mammals (Harris 2009, Jones et al. 2008, Meyer and Butaud 2009, St Clair 2011, Wanless et al. 2007). But in spite of this potential threat, studies with these invaders in the country are still scarce (e.g. Sarmento et al. 2014, Panti-may et al. 2016, Richardson et al. 2017) and although there are some punctual reports of non-native rodents being captured (e.g. Tonini et al. 2010, Cherem et al. 2011), there is few information regarding the occupancy pattern of these species in such complex environment as Brazilian forests.

The Atlantic Forest covers the whole Brazilian coast and is considered a biodiversity hotspot (Myers et al. 2000), this rainforest is the first biome that rats and mice will encounter after their arrival and in this work we aimed to understand how invasive rats and mice are affected by Atlantic Forest landscape. Knowing how deep into the forest these rodents are reaching can give us a glimpse of how threatened Brazilian native species are. Besides we also aimed to identify other elements associated with rats and mice occupancy and abundance in port city settling. This information will allow the improvement of measures of control against the establishment and spread of invasive rodent species in Paranaguá and potentially in other port cities.

Methods

Study area

The study was conducted in Paranaguá (25° 31′ S, 48° 30′ W), a port city with population of 153.600 and area of 827 km^2, localized in Paraná state, South of Brazil (Fig. 1). Paranaguá is surrounded by a forest continuum of Atlantic Forest (Ombrophilous Dense Forest) and has 21,7% of its territory composed by five protected areas. The port, started as a berth in 1872, and then was officially founded in 1935. It has an organized area of 425 km2 and is the third most important in the country moving mainly solid bulk (32 million tons in 2018), but also liquid bulk and containers (ANTAQ 2018; APPA 2019). It is the most important port in South America in relation to soy, corn and bran exports.

To estimate abundance and occupation of invasive rodents in Paranaguá, we selected 18 sampling sites along the city according to their distance from the port towards the forest. Because of their proximity with Paranaguá’s urban environment, the sampling sites inside the forest were made in Palmito State Park (PSP). PSP is an integral protection conservation unit comprising 1782 hectares of native vegetation, composed by Dense Ombrophilous Forest in the Lowland Formation, pioneering formations with marine influence and pioneering formations with fluvio-marine influence (mangrove) (IAP 2019).

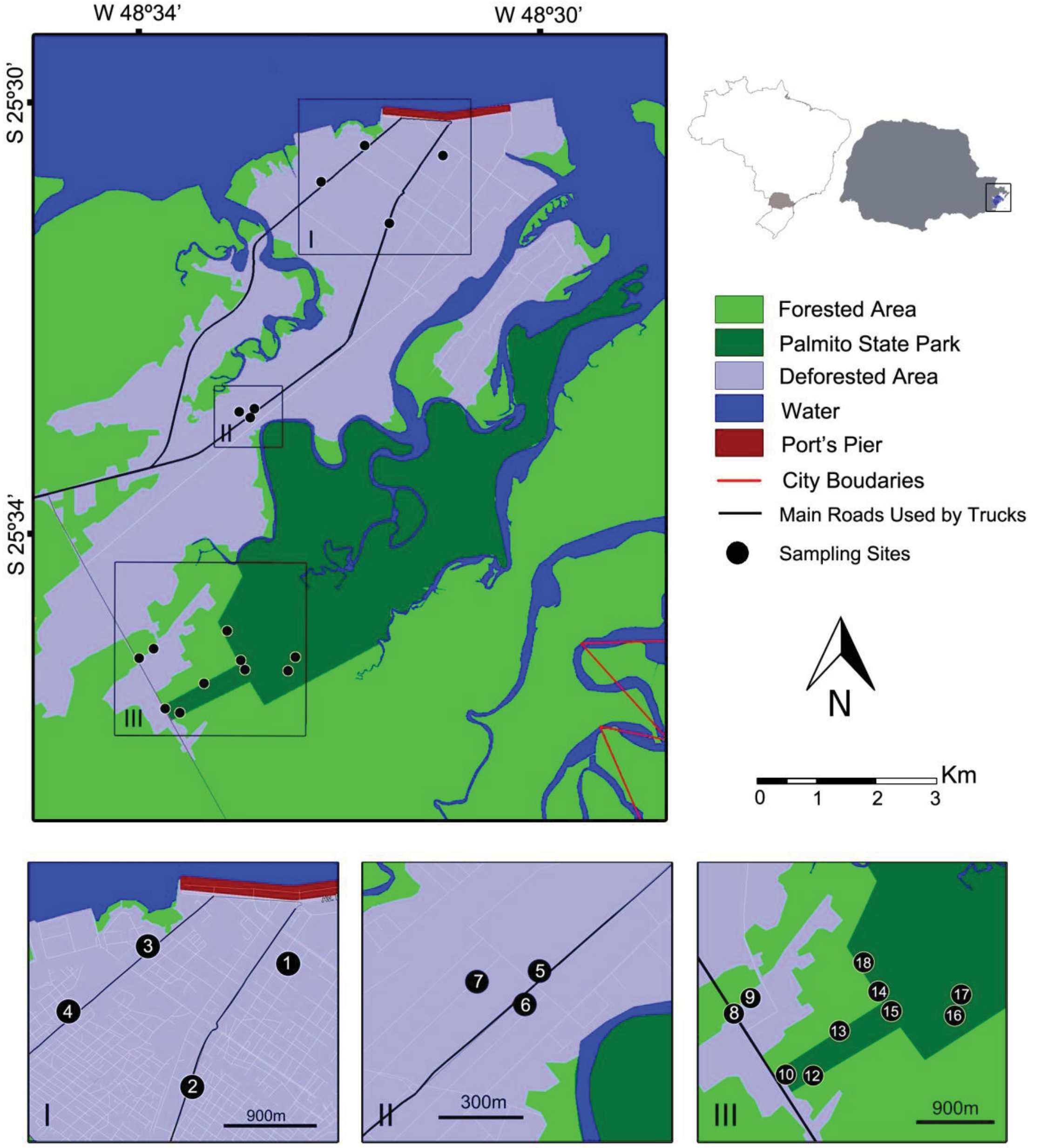

Fig. 1 Location of the study area in Paranaguá, Paraná state, Brazil. Only the area where the vessels dock is represented as port. In III, sampling site 10 and 11 are overlapped.

Data collection

The data collection was made between January 2017 and July 2018 in 9 field campaigns using three sizes of Sherman live traps model (9 cm x 9 cm x 25 cm; 9.5 cm x 9.5 cm x 31 cm; 40 cm x 21 cm x 21 cm) and two sizes of Tomahawk live traps (18 cm x 14.5 cm x 35 cm; 13 cm x 13 cm x 22 cm) in 16 sampling sites.

Live traps sampling followed two designs depending on the space available to set the traps: (1) on sampling sites 2-11 the traps were randomly distributed in an area with an average size of 452m^2 (SD = 85 m^2), and (2) on sampling site 1 and 12-18 traps were placed every 10m in line transects. Once the urban context of some sites did not allow traps to be installed every day, sampling efforts ranged from 35 to 1069 traps.night (Table 1) The traps were baited with a mixture of banana, cornflour, peanut candy and fish oil.

Trap effort (trap.night) applied and general environmental context of each sampling site. LT = Line Transect, RDA = Randomly Distributed in the Area.

Sampling Site	Trap Method	Trap Effort	Context of the Site
1	*Live-trap*/LT	71	Urban Environment
2	*Live-trap*/RDA	156	Urban Environment
3	*Live-trap*/RDA	45	Urban Environment
4	*Live-trap*/RDA	57	Urban Environment
5	*Live-trap*/RDA	45	Urban Environment
6	*Live-trap*/RDA	121	Urban Environment
7	*Live-trap*/RDA	142	Urban Environment
8	*Live-trap*/RDA	158	Edge
9	*Live-trap*/RDA	1.069	Edge
10	*Live-trap*/RDA	386	Edge
11	*Live-trap*/RDA	35	Edge
12	*Live-trap*/LT	38	Forest
13	*Live-trap*/LT	253	Forest
14	*Live-trap*/LT	273	Forest
15	*Live-trap*/LT	312	Forest
16	*Live-trap*/LT	351	Forest
17	Pitfall	362	Forest
18	Pitfall	340	Forest
Total		4.214	

In two forests sampling sites (17 and 18) we used pitfall traps. They were installed as straight lines, containing 10 buckets (60L) spaced 10m from each other.

All captured individuals were processed following the procedures approved by UFPR Ethics Committee (CEUA Nº1211) and by the Brazilian environmental agency (License Nº 23102-1 SISBIO).

Covariates and Associated Hypotheses

We used seven covariates to model abundance and occupancy probability of the invasive rodents. In order to test our hypothesis that abundance and occupancy probability of invasive rodents could be associated with the forest, we measured distance from forest edge (edge) and forest cover percentage within radius of 1000m (forest). To test if the port has a significant role as entrance door to rats and mice, the distance from the pier where the vessels dock (port) was used as a covariate as well. Also, to test if the routes of dispersion would affect these rodents occupancy, we measure the distance from the main roads used by trucks to access to the port area (truck) and distance from the nearest road (road). Additionally, abundance of human edifications within radius of 100m (edification) and distance from the nearest human edification (edification_edge) was used to evaluate human association with the target species.

For the covariate distance from forest edge, negative values were attributed to sampling sites inside the forest and positive values to those outside of it, and the nearest forest was considered any fragment with more than 5 ha. To measure abundance of human edifications within radius of 100m and distance from the nearest human edification, we only considered houses and buildings once these are presumed to be directly related to human occupation. Therefore, roads, truck yards and abandoned lands were not counted.

A central point of the area/transect was chosen to take measures in each sampling site. All covariates were measured using satellite images taken in 2018, available in Google Earth Pro software (7.3.2.5491 version).

Data Analysis

Occupancy modelling accounts for imperfect detection by interpreting a series of detection / non-detection events as arising from an interaction between a species' probability of patch occupancy (Ψ) and its detection probability (P). A detection event occurs when the site was occupied, and the species was detected (Ψ × P). A non-detection occurs because (1) the species was present but not detected (Ψ × [1–P]), or because (2) the species was not present and therefore was not detected (1–Ψ). Moreover, the maximum likelihood method, used in this occupancy modeling approach, was considered to give the least biased estimates of occupancy and detection probabilities in a series of alternative methodologies (Wintle et al., 2004).

In this context, we implemented Royle-Nichols occupancy models in program PRESENCE (Royle and Nichols 2003) to estimate abundance of both rodent species (*Mus musculus* and *Rattus norvegicus*). These models assume that heterogeneity in detection among sites is the result of underlying differences in abundance (Royle and Nichols 2003). This analysis estimates an index (λ) of mean abundance per site, thus allowing us to assess differences in both species abundance between the 18 sampling sites. The Royle/Nichols occupancy model assumed that: 1) the number of animals at a site followed a defined spatial distribution for which λ-hat indicates the mean abundance across all transects; and 2) the probability of detecting animals in each site was related to the species' inherent detection probability, r-hat, and the total abundance.

We were also interested in how the habitat covariates affect each species' occupancy and detectability. Therefore, Single–species, single–season occupancy models were created for each species using the "Unmarked" package in Program R (Fiske and Chandler 2011). We classified each sampling trip into 9 occasions based on Mackenzie et al. (2006) approach to construct a detection history. We constructed a set of candidate models for each species, which were selected by a priori hypotheses based on three different approaches: (1) considering occupancy probability and detectability as constant across all sites, (2) considering the variation in occupancy as a function of the measured covariates, and (3) considering only detectability as a function of the measured covariates. Top models were selected using Akaike's information Criterion adjusted for small sample size (AICc). All models with a ΔAICc value < 2 were considered equivalent. We also used the weight (AICcwt) for each model, which corresponds to the amount of evidence in favor of a given model, to choose the best model that we used for testing our hypotheses.

Results

A total effort of 4.214 traps.night (Table 1) resulted in 33 captures of *Rattus norvegicus*, 16 of *Mus musculus* and 1 *Rattus rattus.* Due to low detection of *Rattus rattus*, this species was excluded of abundance and occupancy analysis. No exotic species was captured inside the forest environment.

Royle-Nichols occupancy Models

A Royle/Nichols model with the forest cover as covariate best explained mean transect abundance of both rodent species (wi = 0.82; Table 2).

Table 2. Royle/Nichols Occupancy Model Royle; Nichols (2003) selection for mean sampling site abundance (λ) for both rodent species *Mus musculus* and *Rattus norvegicus* based on AICc values for Paranaguá/PR. AICc = Akaike's Information Criterion, ΔAICc = difference in AICc relative to the smallest value, AICcw= AICc weight, K = number of parameters.

Model	**AICc**	**ΔAICc**	**AICcw**	**K**
Mus musculus				
λ(forest)r(.)	258.25	0	0.82	3
λ(.)r(.)	263.54	5.29	0.09	2
λ(forest)r(forest)	265.36	7.11	0.02	4
λ(.)r(forest)	269.12	10.87	0.01	3
Rattus norvegicus				
λ(forest)r(.)	159.42	0	0.82	3
λ(.)r(.)	164.25	4.83	0.09	2
λ(forest)r(forest)	167.52	8.1	0.02	4
λ(.)r(forest)	169.69	10.27	0.01	3

The estimated population size (N-hat) for *Mus musculus* to the whole sampled area was 140 ± 12 individuals (95% CI = 128-152 individuals) and for *Rattus norvegicus* was 82 ± 9 individuals (95% CI = 73-91 individuals). The estimated mean abundance for both species was negatively affected by the forest cover, with λ-hat dropping with higher forest cover sites (Fig. 2).

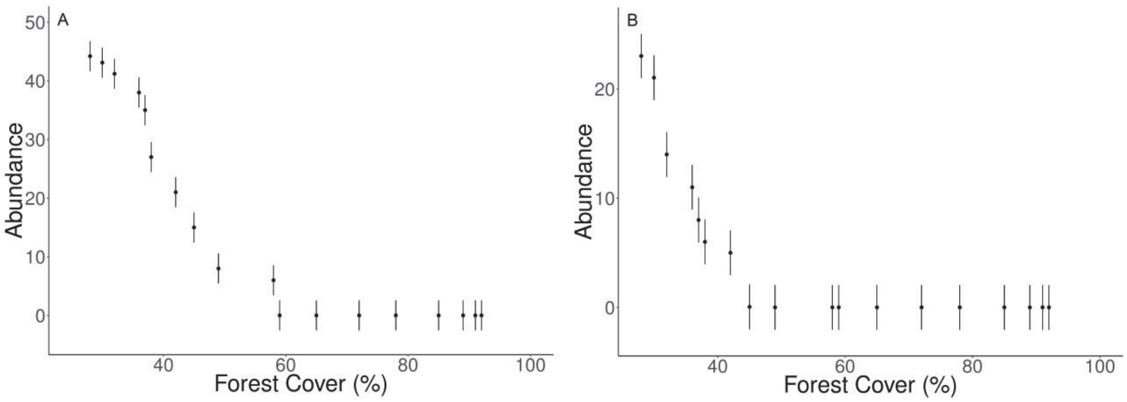

Fig. 2 Variation of abundance as function of the 'best-fit' covariate according to Royle/Nichols model. *Mus musculus* abundance in relation to percentage of forest cover within a 1000m radius (A); *Rattus norvegicus* abundance in relation to percentage of forest cover within a 1000m radius (B).

Single-species occupancy models

For clarity, we only present the top 10 models, based on ΔAICc, for occupancy of *Mus musculus* and *Rattus norvegicus* in Table 3.

Mus musculus was observed at 3 of the 18 sampling sites, resulting in a naïve occupancy probability of 0.45. Estimated mean occupancy and detection probabilities were 0.51 ± 0.06 and 0.35 ± 0.03, respectively. The 'best-fit' models described occupancy probability as an additive function of the site's abundance of human edifications, distance from forest edge, and forest cover, and detection probability as constant (Table 3).

Occupancy of *M. musculus* was higher at sites with more human edifications (Ψ dropping from 0.97 ± 0.03 to 0 ± 0.02; Fig. 3A) and at sites closest to forest edges (Ψ dropping from 0.89 ± 0.03 to 0 ± 0.04, respectively; mean ± SE; Fig. 3B). Occupancy was negatively affected by the forest cover, with probabilities ranging from Ψ = 0.98 ± 0.03 to Ψ = 0 ± 0.03 (Fig. 3C).

Table 3. Single-season occupancy and detectability models for *Mus musculus* and *Rattus norvegicus* in Paranaguá, Brazil. Covariates include: distance from forest edge (edge); distance from truck access (truck); forest cover (forest); distance from the closest road (road); abundance of human edifications (edification). Ψ = occupancy, *p* = detection, ΔAICc = difference in AICc relative to the smallest value, AICcw = Akaike weight.

Model	**ΔAICc**	**AICcw**	**N parameters**
Mus musculus			
Ψ(edification;edge) p(.)	0.00	0.25	4
Ψ(edge;forest) p(.)	1.02	0.23	4
Ψ(edification;edge;forest) p(.)	1.26	0.21	5
Ψ(edge) p(.)	3.69	0.18	3
Ψ(edification;forest) p(.)	6.25	0.08	4
Ψ(.) p(.)	8.17	0.03	2
Ψ(edification) p(.)	10.63	0.01	3
Ψ(edification;edge;forest;road) p(.)	13.45	<0.01	6
Ψ(forest) p(.)	14.25	<0.01	3
Ψ(edification;edge;road) p(.)	14.67	<0.01	5
Rattus norvegicus			
Ψ(truck; forest) p(.)	0	0.72	4
Ψ(truck) p(.)	3.69	0.12	3
Ψ(forest) p(.)	5.56	0.08	3
Ψ(truck;road) p(.)	9.25	0.05	4
Ψ(truck;forest;road) p(.)	11.25	0.02	5
Ψ(truck;forest;edification) p(.)	13.65	<0.01	6
Ψ(truck;edification) p(.)	15.25	<0.01	4
Ψ(forest;road) p(.)	15.68	<0.01	4
Ψ(.) p(.)	18.25	<0.01	2
Ψ(truck;forest;road;edification) p(.)	18.36	<0.01	6

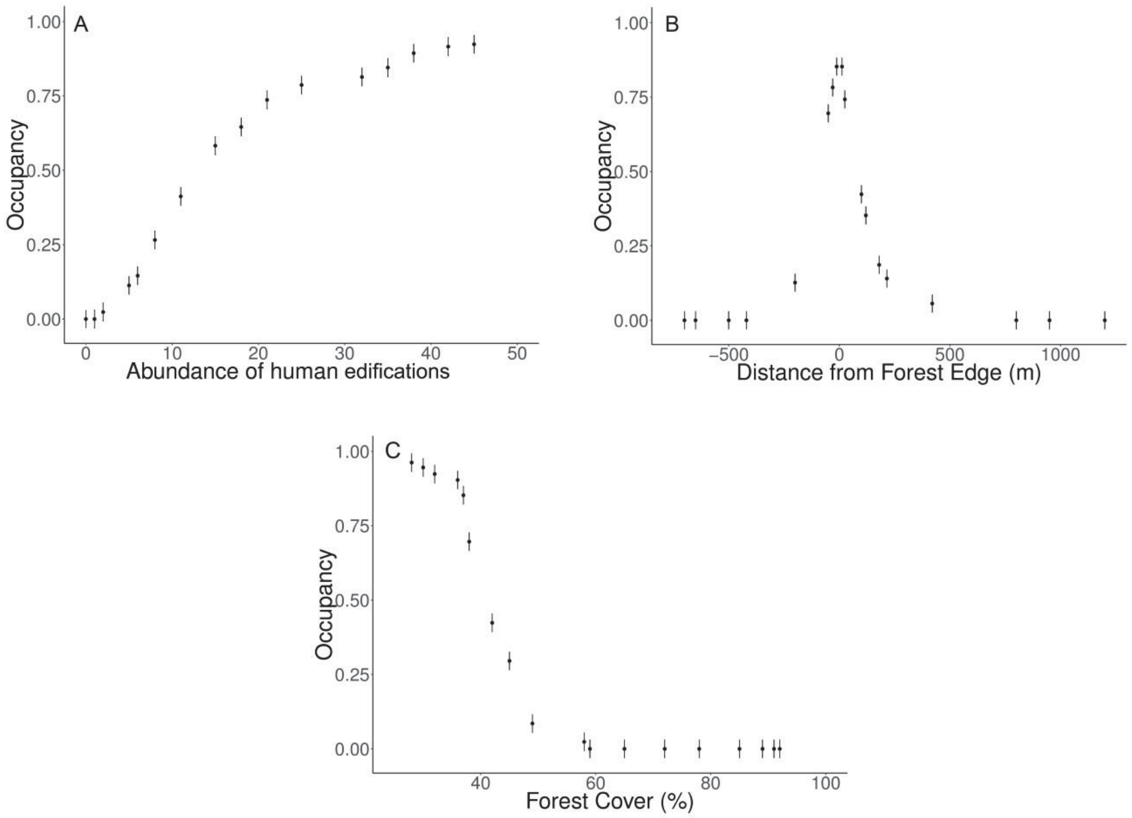

Fig. 3 Occupancy probability by *Mus musculus* according to the 'best-fit' models. Relation between occupancy and the abundance of human edifications within radius of 100m (A, relation between occupancy and distance from forest edge (B), negative values were attributed to sampling sites inside the forest and positive values to those outside of it; relation between occupancy and percentage of forest cover within radius of 1000m (C).

We observed *Rattus norvegicus* at 6 of 18 sampling sites, resulting in a naïve occupancy probability of 0.34. Estimated mean occupancy and detection probabilities were 0.42 ± 0.04 and 0.24 ± 0.03, respectively. The 'best-fit' model described occupancy probability as a function of distance from truck access and forest cover. Detection was best modeled as constant with no effect of the measured covariates. Occupancy was higher ($\Psi = 0.98 \pm 0.02$) at sites closest to truck access (Fig. 4A) and ($\Psi = 0.96 \pm 0.03$) at sites with the lowest forest cover (Fig. 4B).

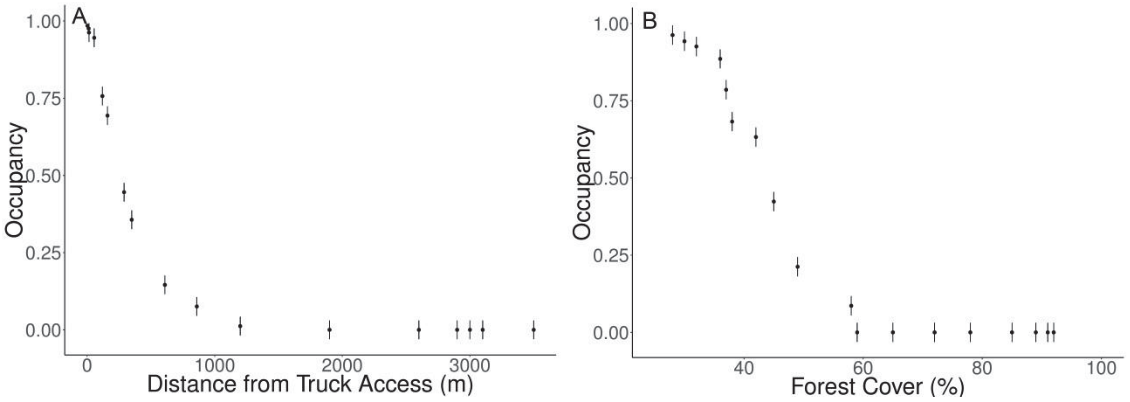

Fig. 4 Occupancy probability by *Rattus norvegicus* according to the 'best-fit' models. Relation between occupancy and distance from road used by trucks to access the port (A), relation between occupancy and percentage of forest cover within radius of 1000m (B).

Discussion

The percentage of forest cover variable presented a negative relationship with the occupancy rate and abundance of both species. The forest cover seems to strongly affect *R. norvegicus,* as its abundance drops to zero when the forest cover reaches 45%. *M. musculus* has its abundance slightly less affected reaching zero abundance when the forest cover is 59%. Regarding the occupancy probability, for both species it reach zero when the forest cover within radius of 1000m is 59% or higher. Gatto-Almeida et al. (2016) performed a species survey in a natural reserve, also located close to the urban environment in Atlantic Forest biome, and with similar characteristics to the area of the present study, it had sampling effort of 6.633 traps.night and also did not recorded invasive species inside the forest even when a deactivated landfill inside the protected forest was sampled.

In contract, in Salzburg, Austria the Norway rats were found to be actually associated with patches containing multi-layer vegetation (ground vegetation, bushes and trees) and the authors found low trapping success in patches without any vegetation (Traweger et al. 2006). Similarly, mice have been largely recorded inside forests in New Zealand although they seem to be more common in disturbed zones (King et al. 1996, Walker et al. 2019). Our analysis suggests strong factors negatively affecting these two species presence near to areas with great forest cover.

It has been proposed that the presence of the red squirrel, *Sciurus vulgaris,* is responsible for the absence of *R. rattus* in Britain's forests, and the absence of specialist arboreal rodents in New Zealand is what allowed the ship rat to occupy forests (King et al. 2011). Once the Atlantic Forest possesses high diversity of small mammals (Paglia et al. 2012), competition could explain why invasive rodents were not captured inside Paranaguá's forests, but it does not explain why they have low abundance in areas outside but near the forests.

The observed low abundance and occupancy of exotic rodents in areas with high proportion of forest cover is probably related with predator presence, which does not exist in New Zealand, for example. It has been shown avoidance behavior in *R. norvegicus* towards different mammals (Yin et al. 2011). In Paranaguá, the bigger proportion of forest cover could be related to the larger number of potential predators in the area, which could lower mice's and rat's densities and inhibit their occupancy. In fact, bone fragments of *M. musculus, R. rattus* and *R. norvegicus* are commonly identified in regurgited pellets of the Barn Owl (*Tyto furcata*) in Brazil (Scheibler and Christoff 2004, Lemos et al. 2015). *R. rattus* and *M. musculus* were also recorded as preys of *Cerdocyon thous*, a small canid common in South America that frequently occupy forest edges and human altered areas (Dotta and Verdade 2007, Gatti et al. 2006, Pedó et al. 2006). Both predator species could leave the forest shelter to hunt and hence decrease the density of invasive rodents in the surrounding area. Besides, these two species are not the only potential predators to rats and mice, the Atlantic Forest possess at least 5 species of wild felines (Paglia et al. 2012) and 70 birds of prey (ARB 2019) that could present a threat to these invaders in case they come too close to their habitat.

Unfortunately, most cities do not have big remnants of Atlantic forest close to the urban environment like Paranaguá. According to Brazil's Native Vegetation Protection Law (Nº 12.651/2012) any rural property located in Atlantic Forest Biome is obligated to preserve 20% of the native vegetation, but our results suggest this amount is inadequate to control invasive rodent populations.

A recent study remotely sensed the vegetation cover of Brazilian Atlantic Forest and revealed 28% of remaining native vegetation cover instead of the 11-16% that were estimated before, but this increase is due to identification of fragments and secondary forests not mapped previously (Rezende et al. 2018). A fragmented landscape is probably not so efficient in controlling rats and mice invasion but (Rezende et al. 2018) just shown a large potential to natural regeneration of Atlantic Forest so the forest cover of this biome gets increased. If the existing legal debt of Atlantic Forest vegetation was restored, Atlantic Forest cover would reach 33-35% of its original area (Rezende et al. 2018) and this biome could represent a natural barrier to the invasion of exotic rodents in Brazil's coast.

Brazil has high species richness regarding small mammals (Paglia et al. 2012), and the traditional bait poisoning method used to control population of rats (Courchamp et al. 2003) could dangerously affect the native species in contact areas. In this context, the Atlantic Forest reveals a remarkable ecosystem service that has been underestimated in studies of eco-services valuation (e.g. Costanza et al. 1997, 2014, Groot et al. 2012). Our data show, at first time, that this tropical forest has a considerable negative impact on occupancy and abundance of two invasive rodents, hence can be used as an alternative/complementary method to contain exotic rat invasions. Rats are known to cause loss of billions of dollars every year (Pimentel et al. 2000), and their natural control, by the Atlantic Forest, has a significant value as ecological service that should be considered in future studies of valuation, and in conservation policies to be implemented.

In addition to forest cover the occupancy analysis of the House mice also showed its preference to occupy regions with higher abundance of human edifications (within 100m radius). In contrast with *R. norvegicus* that usually nests in burrows in the soil (Traweger et al. 2006, Varnham 2010), *M. musculus* tend to spend more time in places with complex ground-level, or at least with overhead cover in cases of open area with no ground level structure (Jensen et al. 2003), those criteria are attended by human constructions or forests. Thus, measures to control mice population in Paranaguá should focus in encourage population to keep their houses under constantly surveillance, especially backyards that can present a complex ground-level, ideal to mice occupancy.

Furthermore, a third covariate presented positive association with mice occupancy: distance to the edge of the forest. Mice had preference for areas close to the edge of the forest presenting a bell curve that reaches its peak (Ψ = 0.85 ± 0.03) 12m far from the edge and 12m inside the vegetation. This result does not contradict those related to the forest cover; the covariate “edge of the forest” does not reflect quantity of forest but proximity of natural shelters. Paranaguá has a lot of small fragments and jagged edges that were considered as the closest edge of sampling sites but due to its small size or degraded status are not preferable spots to predators to occupy. This result reinforces our hypothesis that the negative association with forest cover is more related with predator presence than with capability of colonize the forest environment. This also makes *Mus musculus* rise as bioindicator of edge effect.

Regarding *R. norvegicus,* occupancy analysis also shown positive association with roads used by trucks to access the port. Trucks can accidentally transport rats just like vessels do in water (Sanu and Newport 2010), and this kind of dispersion can be especially frequent in a port city where trucks are constantly loading cargos and containers that can potentially be infested from overseas transport. It is important to remember that we tested if distance to any road would be an important covariate, but only specifying the two main roads that give access to the port area this covariate presented significance. In Paranaguá there are two main roads used by trucks to access the port area. It is common to register big amounts of grain that falls from loaded trucks along these roads, mainly of soybean and maize (S1 Appendix), which provides easy access to food to rats, and explain occupancy probability reaching Ψ = 0.98 ± 0.02 in the closest sampling site to the road. Therefore, the results suggest the first control measure to be implemented by local authorities should aim the region close to these two main roads where the occupancy probability by invasive rats is the highest. Additionally, we advise other port cities to keep their main access to the port under surveillance once this indirect association is probably not an exclusive trait of Paranaguá.

As it has been shown (King et al. 2011), our results showed that even invasive species such as rats and mice, that have been able to adapt to most places around the world, can present different outcomes depending of the environment and the species they encounter. Understanding how these invaders interact with local environment can be the key to develop efficient and directed control/eradication programs, and that is why studies like this in other regions around the world should be encouraged.

Finally, the distance to the pier was not a significant covariate which means our hypothesis of higher occupancy and abundance near to the pier, where the rodents have higher probability to arrive, was rejected. This result can be due to two causes: (1) if the port is the entrance door for invasive rodents, at the moment, the established population is more representative than new immigrants, or/and (2) the control measures implemented in the vessels dock area are working and should be extended to the rest of the city. What we know for sure is that rats and mice are spread out through the city, and even though prevention measures to reduce the risk of new introductions are still important, our results suggests that Paranaguá should focus in implement comprehensive and continuous measures to reduce the population of invasive rodents.

South America has been identified as having a low index of publications on non-native species (Speziale et al. 2012) and in Brazil only 15 of the 143 papers published in this area, between 1999 and 2015, had mammals as objects of study (Zenni et al. 2016). This is the first work to evaluate the abundance and distribution of invasive rodents in a Brazilian city. In addition, the methods used in this paper are usually employed in studies that aim to estimate population abundances and understand native mammals, so better conservation strategies can be developed (Ferreguetti et al. 2018, 2017, Thorn et al. 2011). Thus, we also used, for the first time, the employment of occupation modeling to develop management strategies to control populations of invasive rodents.

Conclusions

Paranaguá has a privileged ecological situation where the whole city is surrounded by a forest continuum that seems to be confining the rats and mice to the urban environment and containing their spread to outside the city. Our work has shown that rats are mainly far from the forest and close to the main access to the port, pointing the exact area where management measures should be implemented. The proximity of *M. musculus* to forest edges reinforce that keeping the integrity of the forest is key to maintaining the ecosystem service of the Atlantic Forest as a natural barrier to these rodents.

Studies that continue this work, detecting possible contact areas between exotic and native rodent species, as well as studies with population approaches to understand the pattern dispersion inside the city may be fundamental for better understand their behavior and thus a better refinement of management strategies.

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Supplemental Material 1. Fallen grains along one of the main roads used by trucks to access port area (A-C).

5. CAPÍTULO II. ASSESSMENT OF DISPERSAL AND POPULATION STRUCTURE IN NORWAY RAT POPULATIONS IN A SEAPORT SETTING

Assessment of dispersal and population structure in Norway rat in a seaport setting

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Abstract

Seaports are considered introduction epicentres to invasive alien species, like rodents, the most widely introduced vertebrates to accompany humans. After its arrival in a new environment, linear features in the landscape, like roads facilitate their spread and colonization. We aimed to genetically characterize *R. norvegicus* population along an important road used by trucks to access the port area in Paranaguá city. We genotyped 11 microsatellite loci of 71 individuals and used diversity indexes, Bayesian clustering and genetic assignment of individuals to investigate gene flow between the three sampled populations. Our results revealed a pattern of gene flow different of the expected stepping-stone model, clustering the two further away populations (Port and Area 3). The intermediate population expected to have more contact with the others was the least diverse regarding number of alleles and allelic richness. We propose that the observed outcome is due to natural dispersal along the corridor being lower than human-mediated transport. The area further from the port presents a gas station often used as parking lot by trucks that are the most probable mean of transportation for the species. Therefore, a more efficient cargo surveillance should improve the contention of Norway rat dispersal, not only for biosecurity but also for sanitary reasons.

Key-words: human-mediated dispersal, microsatellite, road, port city

Introduction

Biological invasions are considered a leading threat to biodiversity around the world and they occur when species are intentionally or accidentally introduced outside of their native or historic range, and successfully spread in the new environment (Levine, 2008). Seaports are considered introduction epicentres to invasive alien species (IAS) (Drake and Lodge, 2004; Miller et al., 2016) and that is why ports usually implement surveillance measures to control the establishment and spread of new migrants such as containment, biocontrol, use of chemicals and harvesting of the invasive species (Gren, 2008).

Paranaguá port has a vector proliferation control program that aims to reduce and control pigeon, rat and mice population by using poison bait (for rats) and bird repellent (for pigeons). In addition, to reduce the food availability, fallen grains are daily swept in the primary area and its surroundings (APPA, 2016).

Rodents are considered the most widely introduced vertebrates accompanying humans (Drake and Hunt, 2009) and cause several negative impacts on native biodiversity (Varnham, 2010) and economic loss, through destruction of grains and stocked materials (Pimentel et al., 2000), and transmission of numerous diseases to humans (Himsworth et al., 2013). These animals are particularly problematic because of their capability of travelling long distances hidden in human transportation vehicles, high reproductive rate, and their capability of exploiting a wide range of food sources and live in the most different ecological conditions (Varnham, 2010).

If an IAS passes through the control measures after its arrival in the new environment, linear infrastructures in the landscape, like roads, railways and canals can facilitate their spread and colonization (Brown et al., 2006; Cameron and Bayne, 2009). In Paranaguá there is a positive association of Norway rats (*Rattus norvegicus*) with the main road used by trucks to access the port area (unpublished data). In this study we aimed to genetically characterize *R. norvegicus* population along that road and through genetic assigment of individuals evaluate how effective are the measures of control implemented in the port area for keeping the rats from spreading towards the rest of the city.

Material and Methods

Study Area

The study was conducted in Paranaguá municipality (25° 31′ S, 48° 30′ W), a port city with a population of 153.600 and 827 km^2 area, located in Paraná state, South of Brazil (Figure 1). The port works since 1872 when was used only as berth, and it was officially founded in 1935. It has an organized area of 425 km2 and is the third most important port in the country moving mainly solid bulk (32 million tons in 2018), but also liquid bulk and containers (ANTAQ 2018; APPA 2019)

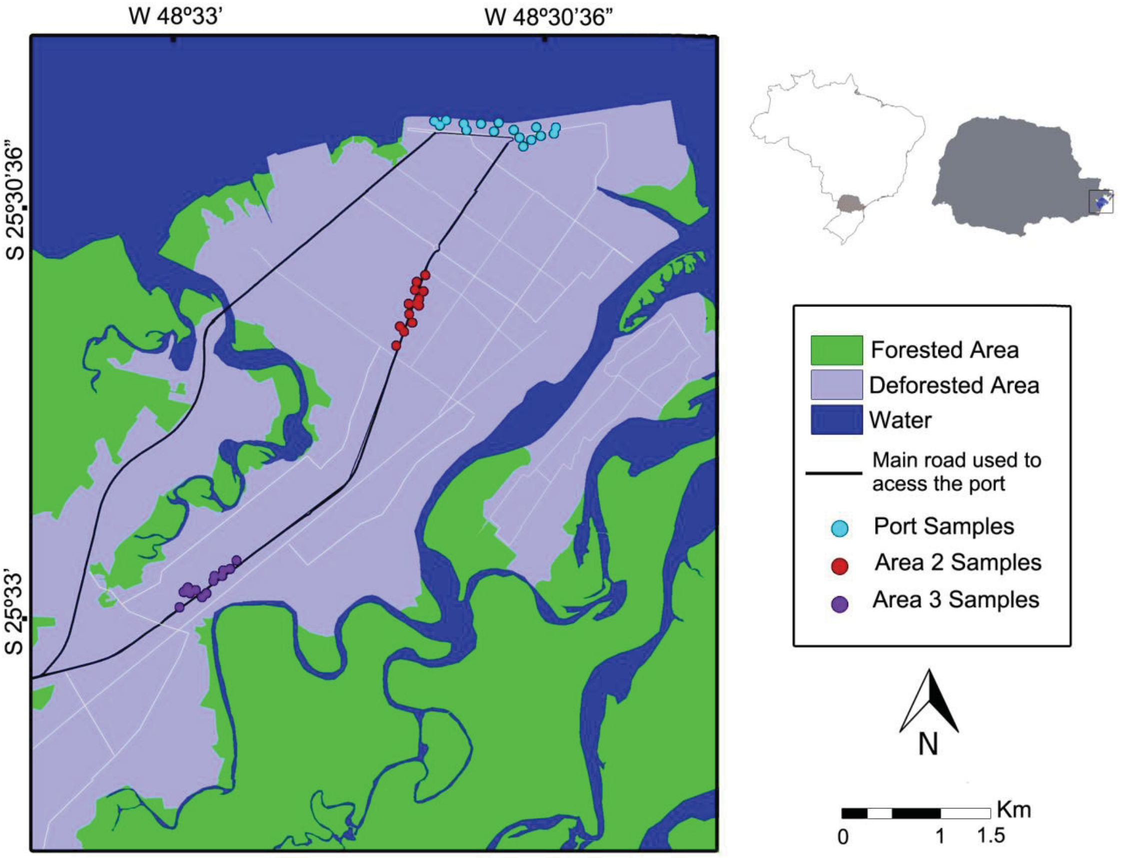

Figure 1. Location of the study in Paranaguá, Paraná state, Brazil. Dots only represent the approximated location where each sample was collected.

Sample Collection

Norway rat tissue samples were collected from three locations across the main road used by trucks to access the port area, which we *a priori* treated as population units. Some samples came from rat carcasses found in the areas and others came from captured animals. We performed 9 field campaigns between January 2017 and July 2018, and applied a total effort of 535 traps.night. Rats were captured in live traps (three sizes of Sherman model 9 cm x 9 cm x 25 cm; 9.5 cm x 9.5 cm x 31 cm; 40 cm x 21 cm x 21 cm and two sizes of Tomahawk model (18 cm x 14.5 cm x 35 cm; 13 cm x 13 cm x 22 cm) and euthanized for tissue collection following the procedures recommended by UFPR ethics committee (CEUA Nº1211).

We intended to see how intensively the “population units” along the road were connected with the port (the entry door of new migrants). Therefore, the first sampling site was the port. Through the establishment of a partnership with the port administration (Administração dos Portos de Paranaguá e Antonina – APPA), we were able to access the carcasses reaped inside the port area by the zoonosis control team and installed live traps in areas where we had free access. For comparison of genetic diversity along the road, rats were sampled in an area 2 km away from the port (Area 2) characterized by the presence of wasteland, residences and a big storage courtyard in the surroundings. At last, a third area (Area 3) 6 km away from the port was selected to be sampled and had as main feature, the presence of a gas station where trucks park to stay overnight, and also had wasteland and residences in the surroundings. Both Area 2 and 3 had extensive zones with natural earth soil parallel to the road where traps were set and the carcasses were found.

The captures were authorized by the Brazilian environmental agency (License Nº 23102-1 SISBIO).

DNA extraction, genotyping and sequencing

Genomic DNA was extracted from muscle tissue samples preserved in ethanol. We used 11 microsatellite markers developed by Jacob et al., 1995 from *Rattus norvegicus*: D12Rat4, D5Rat83, D7Rat13, D9Rat13, D10Rat20, D16Rat81, D11Mgh5, D15Rat77, D2Rat234, D18Rat96 and D20Rat46. Markers were chosen from different chromosomes to avoid physical linkage. The M13 fluorescent labelling protocol (Schuelke 2000) was used to amplify the loci and incorporate the fluorescent dyes 6-FAM and NED (Integrated DNA Technologies®).

The polymerase chain reactions (PCR) were performed in 15 µl volumes containing 2 µl of extracted DNA (>60ng DNA/ µl), 1 X Buffer, 1.5mM $MgCl_2$, 10mM dNTPs, 0.1 µM of forward primer, 0.2 µM of each reverse and M13-labeled primer and 1U Taq. The thermocycler conditions were: initial heat activation of 94ºC for 5min, 25 cycles of 94º for 45s, 55ºC for 50s and 72ºC for 50s, followed by 8 cycles of 94º for 45s, 53ºC for 40s and 72ºC for 50s, and final extension of 72ºC for 15min.

PCR products were mixed with 0.4 µL of GeneScan™ 600 LIZ® (Applied Biosystems) and 7,6 µL of Hi-Di Formamide (Applied Biosystems), this mixture was submitted to heat shock treatment, 95ºC for 5min, 4ºC for 5min. Genotyping runs were performed on an ABI3500 (Applied Biosystems) automated sequencer and analysed using GENEIOUS prime version 2019.0.4 (https://www.geneious.com) including the Microsatellite Analysis External Plugin version 1.4.6 (Biomatters Ltd.)

Data analysis

The software Micro-Checker version 2.2.3 (Van Oosterhout et al., 2004) was used to test for the presence of null alleles, using Bonferroni correction and 3000 randomizations.

The number of alleles (NA), allelic richness (Ar), observed heterozygosity (HO), gene diversity within populations (HS) were calculated with FSTAT 2.9.4 (Goudet 1995) for each population and all individual loci (Table 2). FIS and FST values were calculated according to Weir and Cockerham (1984). Pairwise FST values between populations (Table 3) were calculated and p-values were obtained after 3,000 permutations. A test for departure from Hardy-Weinberg equilibrium was also carried out with FSTAT using 21,000 randomisations.

To approach the question of spatial genetic structure we use bayesian clustering in software STRUCTURE 2.3.4 (Falush et al., 2003; Pritchard et al., 2000). The admixture model with correlated allele frequencies was run with 200,000 Markov chain Monte Carlo interactions for 10 runs, after a burning period of 1,000,000 and number of clusters ranging K = 1 to 4. The output file from STRUCTURE was used in STRUCTURE HARVESTER v0.6.94 (Earl and vonHoldt, 2012) to calculate ∆K values (the rate of change in the log probability of data between successive K values) as suggested by Evanno et al., 2005. Individual membership assignments estimated in STRUCTURE and reorganized by STRUCTURE HARVESTER were aligned by CLUMPP 1.1.2 (Jakobsson and Rosenberg, 2007) and DISTRUCT 1.1 (Rosenberg, 2004) was used to generate the bar plot.

We use the saddlepoint approximation method to genetically assign individuals using the visualization method described by McMillan and Fewster, 2017. This analysis was performed in R 3.5.2 (R Core Team, 2017) with the package geneplot v0.1.0 (McMillan and Fewster, 2017).

Results

Our study included a total of 71 individuals: 25 from the port area, 24 from Area 2 and 22 from the Area 3 and genotyping success was 83.9%. As we expected, Port population was more diverse regarding the number of alleles, allelic richness, gene diversity and observed heterozygosity, and also showed very low F_{IS} (Table 1), which suggests that this is the least inbred population among the three analysed. But curiously, Area 2, only 2 Km apart from the port registered less genetic diversity than Area 3, located 6 Km away from what we considered the entry door to new alleles. Besides, Area 3 showed values of number of alleles, allelic richness and private alleles similar to the Port.

Table 1 Sampling location, number of sampled individuals (N_S), number of alleles (N_A), allelic richness (Ar), private alleles (PA), within population gene diversity (H_S), and observed heterozygosity (Ho derived from H_S and F_{IS} values as $Ho=H_S-(F_{IS}\times HS)$), F_{IS} values (none were significant, based on 21,000 randomisations).

	N_S	Na	Ar	PA	H_S	H_O	F_{IS}
Port	25	79	6,528	18	0,782	0,78389	-0,003
Area 2	24	66	5,430	4	0,708	0,671633	0,051
Area 3	22	76	6,158	13	0,730	0,70191	0,038

Micro-Checker detected excess of homozygotes for D12 locus which can indicate the presence of null alleles. Also, departure from Hardy-Weinberg equilibrium (HWE) was detected for that same locus (Table 2). We performed downstream analysis with and without D12 information and since its presence did not show significant effect, the following results are presented with all 11 loci included. No evidence of false alleles or allelic dropout was detected.

Table *2* Number of alleles (Na), allelic richness (Ar; based on min. sample size of 13 individuals), observed heterozygosity (Ho) and intrapopulation gene diversity (Hs) according to Nei (1987), mean pairwise F_{ST} and F_{IS} for each locus following Weir and Cockerham (1984) (*** indicates significance $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$, based on 21,000 randomisations).

Loci Name	Na	Ar	Ho	Hs	Fst	Fis
D5	12	7.743	0.685	0.757	0.12***	0.105
D10	10	6.935	0.706	0.747	0.028***	0.046
D2	8	6.540	0.768	0.78	0.073***	0.019
D20	10	6.187	0.723	0.731	0.057***	0.028
D11	9	4.396	0.692	0.588	0.117***	-0.181
D7	13	9.516	0.773	0.829	0.069***	0.065
D16	14	9.174	0.782	0.839	0.053***	0.07
D18	11	8.289	0.783	0.782	0.083***	-0.004
D9	7	5.351	0.675	0.709	0.027**	0.043
D15	7	5.280	0.774	0.726	0.01	-0.063
D12	7	4.301	0.548	0.649	0.032**	0.158*
Mean		6.701	0.719	0.74		

According to Wright (1978) criteria, the pairwise Fst values suggested moderate genetic differentiation between Port and Area 2 and between Area 2 and Area 3, while indicates only little differentiation between Port and Area 3 (Table 3), areas farther away from each other

Table 3 Pairwise Fst values (** indicates significance $p < 0.01$ and * $p < 0.05$, after 3,000 permutations)

	Port	Area2
Port		
Area 2	0.0793**	
Area 3	0.0456*	0.0642**

The Bayesian clustering analysis performed in STRUCTURE indicated the most likely number of clusters (K) is equal to two (Figure 2), and confirmed the closeness relation between Area 3 and Port, and the differentiation of these two populations from Area 2. A as expected, the bar plot showed recent (most probably ongoing) gene flow between the three populations.

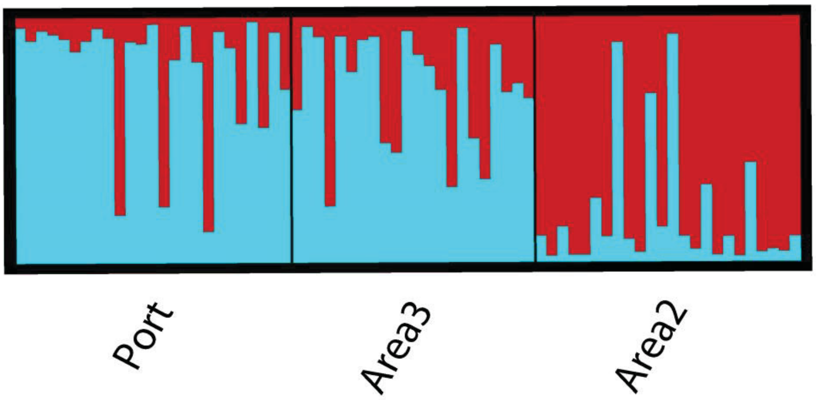

Figure 2 Bar plot of STRUCTURE under admixture model. Estimated proportion of each individual genome to be originated from different populations. The most likely structure of k=2 across 10 replicates was indicated by STRUCTURE HARVESTER.

The saddlepoint approximation method used in geneplot Package corroborated with STRUCTURE and F_{st} values indicated greater genetic similarity between Port and Area 3 population than between Area 2 and Port. The genetic assignment of individuals using Area 2 and Port as reference populations (Figure 3A) revealed that most individuals from Area 3, lie below the thick diagonal line (Port assignment area) that shows equal probability of the individual belong to Port or Area 2.

Regarding port individuals most of them were allocated below the 1% line for Area 2 and therefore have good fit only for ports population. By contrast, almost half of the individuals from Area 2 were nested above the 1% line for Port and between the 1% and 100% line for its own population, meaning that those individuals could be assigned for both populations.

The genetic assignment using Area 3 and Port as reference (Figure 3B) highlighted the relation between those two populations. While five individuals from Port lie above 1% line for Area 3, half of the represented individuals from Area 3 were allocated above the 1% line for Port, meaning those individuals present good fit for both populations.

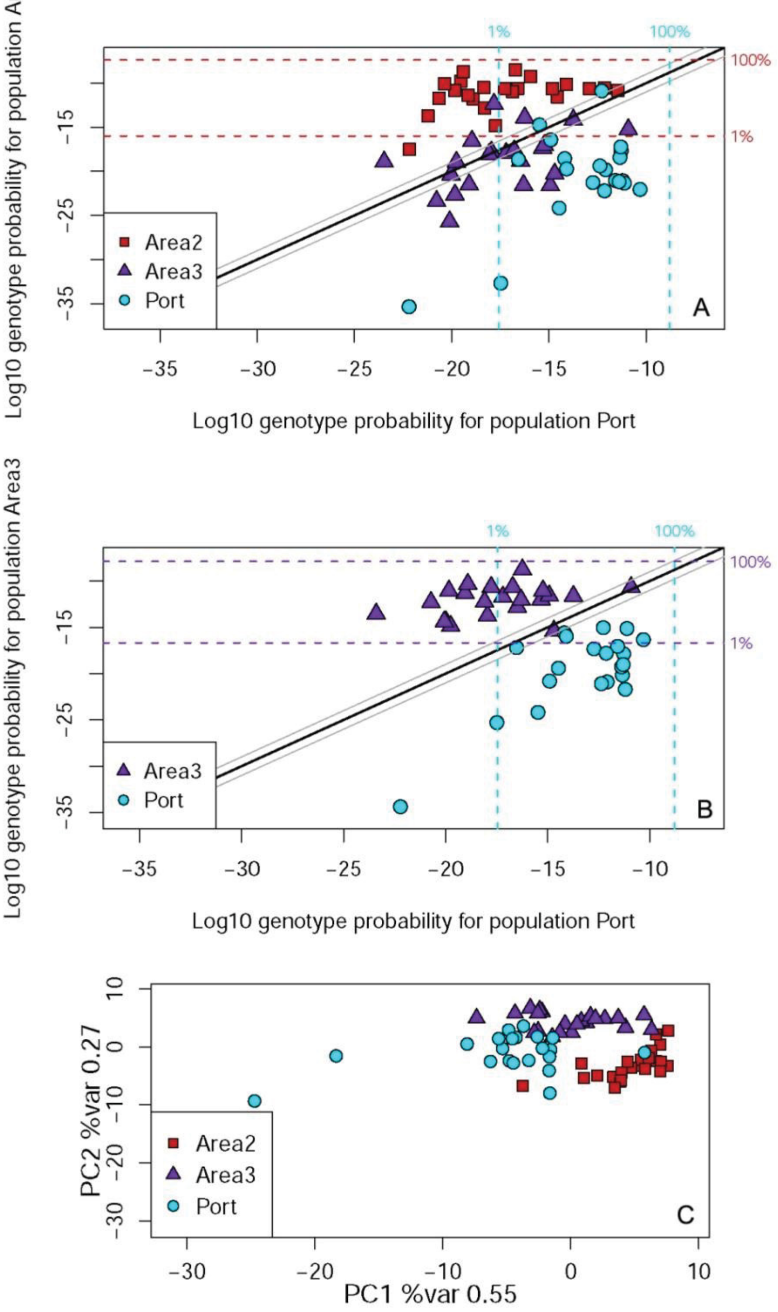

Figure 3 Geneplot for Norway rats in Paranaguá. Genetic assignment for individuals using Port and Area 2 as reference populations (A). Genetic assignments for individuals from Area 3 and Port using those same populations as reference. Individuals from Area 2 are not shown (B). Principal Component Analysis (PCA) using the three populations as reference (C).

Saddlepoint method was also applied in the principal component analysis (PCA) (Figure 3C) where multiple populations can be used as reference, and in agreement with other results, showed higher association between Port and Area 3 than between Area 2 and Port. Although in this analysis we were able to detect possible migrants from Port in Area 2 and vice versa. We also detected two genetically divergent individuals from port population which might represent new immigrants from outside these three populations.

Discussion

Many studies have addressed the question of rat pattern of migration in insular populations (e.g. Abdelkrim et al., 2009; Russell et al., 2009; Russell et al., 2010; Fewster et al., 2011; Pichlmueller and Russell, 2018), but fewer tried to understand the dispersion of rats population in the urban environment. Although eradication is not feasible in the continent, as it is on islands, understanding rat patterns of dispersal in the urban environment can help to develop and refine management and control strategies.

We genotyped samples collected in three areas along one of the main roads used by trucks to access the port area in Paranaguá. In this scale, we expected to observe a pattern of isolation by distance, according to the stepping-stone model of gene flow (Kimura and Weiss, 1964) as reported by Gardner-Santana et al. (2009) for Norway rats population in Baltimore, eastern coast of the USA. But surprisingly, the performed analysis suggests that the two areas farther away from each other (Port and Area 3) are genetically more similar when compared to the intermediate area (Area 2). The reason for this contradictory result is probably related to human-mediated dispersion.

Roads are known key pathways for spread of IAS (Brown et al., 2006; Cameron and Bayne, 2009) and this spread can occur through self-dispersion ways (e.g. displacements by walking) (Brown et al., 2006) or human-mediation, usually associated to long distance dispersal (e.g. transport by vehicles)(VonDerLippe and Kowarik, 2007). Rats also disperse without human mediation, by walking or swimming (Innes et al., 2010; Russell et al., 2010), but *R. norvegicus* seems to exhibit strong site fidelity and low frequency of long-distance dispersal (>500m) in urban environment (Gardner-Santana et al., 2009; Combs et al., 2018). Besides, if self-dispersion was the main mode of gene flow exchange along this road, we would see it happening gradually with Port population presenting higher similarity with its closest area (Area 2) and Area 3 being more related to Area 2, instead of Port (stepping stone pattern).

So, if the results are explained by human-mediated dispersion, why higher genetic similarity is found between Area 3 and Port? Our hypothesis is that the presence of a gas station can be the answer.

Most of Area 3 samples were collected near a gas station where truck drivers park to stay overnight, often with their trucks loaded (either coming out of the port or going into the port area). On land, trucks can accidentally transport rats just like vessels do in water (Sanu and Newport, 2010), specially when the transported cargo is attractive to theses rodents (Paranaguá port is the most important port in South America to soy, corn and bran exports) Any area constantly being accessed by these trucks is susceptible to arrival of new immigrants that would exchange alleles with local population resulting in the clustering observed.

Genetic assignment supports this idea, once half of Area 3 individuals showed good fit to the Port population genotypes. Also, five individuals from Port presented good fit for Area 3 suggesting that gene flow occurs in both ways although is more common alleles from Port arriving in Area 3.

According to McMillan and Fewster (2017) interpretation about geneplot, when individuals from one population show good fit to both reference populations but individuals from the other population only have reasonable fit into their own sampling population, it means that the first population represents a subset of the second, like we registered between Port and Area 3. This pattern was also observed in comparisons between Port and Area 2. In this case, only two individuals from port were assigned above 1% line for Area 2 and half of Area 2 population demonstrated good fit for both populations. The most likely reason for these subsetting results is that Area 2 and Area 3 were founded by Port population, but because of the different intensity of new migrants entering the populations, each one had different outcomes.

Area 2 population seems to have received fewer immigrants so it has lost many alleles due to founder effects, relative isolation and genetic drift. This reflects in lower diversity indexes like number of private alleles, number of alleles and allelic richness. However, Area 3 seems to receive new immigrants more frequently which kept it with high diversity indexes and closer related to its founder population (Port).

Even though we were able to register division between populations in the absence of a geographical barrier we still observed migration occurring between the sites. Our results points that even with control measures being implemented by the port the low F_{ST} values and Bayesian clustering analysis, suggests recent or (more likely) ongoing dispersion between the populations. This affirmation is specially supported by PCA analysis that showed three individuals from Port diverging from its population and one individual from Area 2 clustered with Port population.

Conclusions

Despite the fact that previous studies evaluating *R. norvegicus* in urban environments have found long-distance dispersal (>500m) to occur at low frequency, we were able to register significant human-mediated transport clustering two populations of rats 6 km apart. Trucks are the most probable source of this transportation and can be carrying high number of rats to their destination in mainland's interior. Therefore, we suggest improving cargo surveillance in vehicles, especially when transporting grains in order to decrease the number of migrants coming out of the Paranaguá port, and also to avoid cargo contamination by rats diseases.

Even though trucks are known vectors of IAS, this is the first time that human-mediated dispersal of rats is evidenced in such small scale. Thus, this kind of dispersion should be also considered in the development of port cities management strategies.

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6. CAPÍTULO III. USING GENETICS TO PLAN BLACK RAT (*Rattus rattus*) MANAGEMENT IN FERNANDO DE NORONHA ARCHIPELAGO, BRAZIL

(submetido À revista *Perspectives in Ecology and Conservation*)

Using genetics to plan black rat (*Rattus rattus*) management in Fernando de Noronha archipelago, Brazil

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Abstract

Invasive mammals such as rats are associated with negative impacts on native fauna and their eradication on islands around the world has been proved to result in substantial conservation gains. Fernando de Noronha is an archipelago located off the north-east coast of Brazil and has records of native fauna negatively impacted by introduced rats. We used D-Loop sequences and 14 microsatellite markers to investigate gene flow between three populations of *Rattus rattus* across two islands of Fernando de Noronha. Using both methods we found very great differentiation between the two islands, indicating very low or no gene flow. Our findings suggested that if Rata Island were cleared of rats it would have a low probability of reinvasion occurring from the main island. Thus, we recommend an eradication program be implemented on Rata following the successful program on nearby Meio Island.

Introduction

Marine islands represent 5.3% of Earth’s land and hold 19% of bird species, 17% of rodents and 17% of flowering plants of the world, which means islands are proportionally richer in species than continental areas. However, this high diversity in insular areas has suffered 30 times more extinctions and has 14 times more critically endangered species per km2 than continental areas (Tershy et al., 2015). Invasive mammals are considered as one of the major factors responsible for biodiversity loss on oceanic islands (Caujapé-Castells et al. 2010), and rats are especially significant given their commensal behaviour with humans that allows them to travel long distances and their omnivorous diet that makes them able to exploit many habitats (Drake and Hunt, 2009; Varnham, 2010).

In 2015, more than 580 islands had successfully executed rat eradication worldwide (Russell and Holmes, 2015) and many native species, including invertebrates, birds, mammals and reptiles, are shown to be benefited after invasive mammal eradication (Jones et al., 2016). On Fernando de Noronha there are records of rats preying on turtle nests, and the most common species of invasive rat on the island, the black rat (*Rattus rattus*) (Russell et al., 2018), was possibly one of the causes for the extinction of *Noronhomys vespuccii*, an endemic rodent from Fernando de Noronha (Carleton and Olson, 1999). Besides that, Noronha holds 11 of 14 seabird species occurring on Brazilian offshore islands, some almost restricted to rat-free secondary islets and with small populations that could become extinct soon (Mancini et al. 2016; Russell et al. 2018) and therefore are vulnerable in case of rat invasion.

Rat eradication on Fernando de Noronha would be a complex task but also has the potential to bring significant benefits to local biodiversity and human health (Russell et al., 2018). Russell et al. (2018) recommended that Rata Island could be used as an experimental site for pest eradication to protect and restore breeding seabird colonies. To minimize eradication chances of failure, an increasing number of studies have recommended to perform pre-eradication genetic analysis to define appropriate eradication units (e.g. Abdelkrim et al. 2005; Savidge et al. 2012; Adams et al. 2014). As an example, a genetic assessment of the *Rattus rattus* population on Pearl Island, New Zealand, revealed that an eradication failure was most likely due to reinvaders from an adjacent population (Russell et al., 2010). If the genetic assessment had been performed before the eradication operation this migration between islands might have been more obvious. On the other hand, Adams et al.(2014), performed a pre-eradication genetic analysis on the common brushtail possum (*Trichosurus vulpecula*) population in Dunedin and on the Otago Peninsula, New Zealand, and detected a potential reinvasion pathway. In that case, the authors recommended that the Eastern Peninsula be treated as one eradication unit. The definition of eradication units can help to decide how biosecurity effort may be applied to prevent recolonization from surrounding islands after an eradication is done as one unit.

On Fernando de Noronha the two largest islands (the main island and Rata Island) are separated by 1800 m which is more than black rats are believed to be able to swim (Russell and Clout, 2005) and hence recolonize. However, between those islands there are three smaller islands (Rasa, Sela Ginete and Meio) that might be used as stepping stones and enable gene flow across the islands. This paper aims to investigate gene flow between two putative populations on the main island and a third on Rata Island, assessing population genetics of the most common invasive rat (*Rattus rattus*), and validating the feasibility of the Rata Island eradication strategy proposed by Russell et al. (2018).

Material and Methods

Study Area

Fernando de Noronha is a volcanic archipelago lying 345 km off the north-east coast of Brazil (3º50’S, 32º26’W), composed by 21 islands with total land area of 26 km2 (Figure 1). The main island is 17 km2, and is the only inhabited. The island has a human population around 3,000 residents and receives more than 90,000 tourists every year (MTUR, 2017). About 70% of the main island is a National Park, while the rest is classified by law as environmental protection area, where infrastructure is permitted but controlled. The remaining islands can only be visited under permits of Institute Chico Mendes for Biodiversity Conservation (Silva, 2013a).

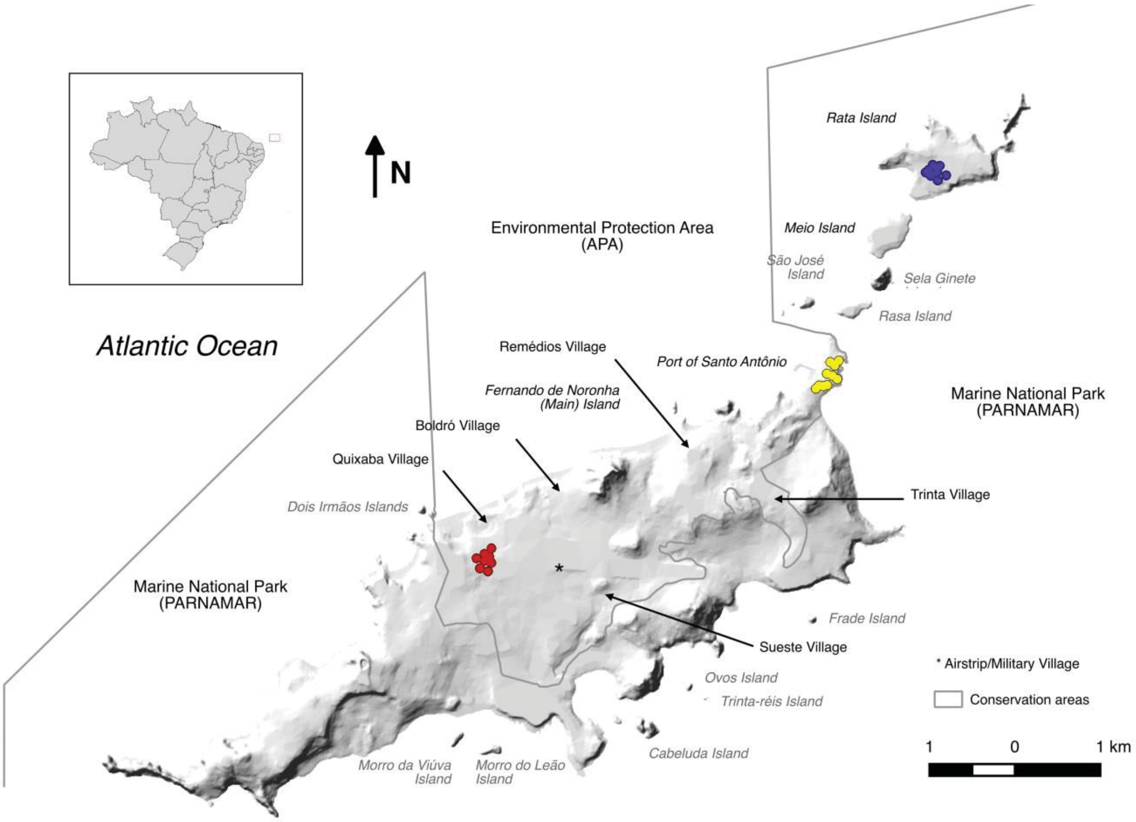

Figure. 1 Fernando de Noronha archipelago map. Sampling sites coloured. Credit: Ricardo Dias.

Our area of interest was the north-east end of the archipelago comprising the main island and stepping stones of Rasa, Sela Ginete, Meio and Rata Island (Figure 1). Rasa Island (5 ha) is low and flat and almost connected to the main island at low tide, but with undercut rock and strong currents remains difficult to access. Sela Ginete (5 ha) is a tall sparsely vegetated granitic column and also treacherous to land upon. Meio Island (17 ha) is low, flat and undercut also like Rasa Island, and in 2017 black rats were successfully eradicated (unpublished data) as a proof of concept for the application of rat eradication methodologies on Fernando de Noronha. Rata Island (89 ha) is a combination of undercut rock but with a main rocky beach exposed to a strong swell, and the forested island gently rises up to a high point (Figure 2).

Figure. 2. North-east view of Fernando de Noronha. At the bottom, the main island and stepping-stones of Rasa, Sela Ginete, Meio and Rata Island at the top. Credit: James Russell.

Sample Collection

Black rat tissue samples were collected from three sites across the archipelago from 2015 to 2018 which we *a priori* treated as population units. Rats were captured in live traps (Tomahawk, 21x21x30cm) and euthanised for tissue collection using Ketamine-Xylazine (K: 90 mg/kg + X: 10 mg/kg SQ) for general anaesthesia, followed by euthanasia using 0.2ml of T-61 (Embutramide 200mg/ml + Mebezonium iodide 50mg/ml + Tetracaine hydrochloride 5mg/ml). We were most interested in connectivity from the north-east point of the main island, around the port where rats might either depart by swimming along the stepping-stones of the north-east chain, or by hitch hiking on boats. Therefore, the first sampling site was the harbour on the main island. For comparison of genetic diversity across the main island rats were also sampled from the village of Quixaba lying in the middle of the main island 4.8 km from the port. At last, rats were sampled on Rata Island. We were not able to obtain samples from Sela Ginete due to its inaccessibility, and no samples were available from Meio Island prior to the rats being eradicated.

DNA extraction, genotyping and sequencing

Genomic DNA was extracted from tails and ears tissue samples, preserved in ethanol using DNeasy Blood and Tissue Kit (Qiagen), following the manufacturer's protocol.

D-Loop mitochondrial region was amplified using EGL4L and RJ3R primers developed by Robins et al. (2007). Each PCR reaction had a final volume of 20 µL, 10 mM Tris HCl pH 8.3; 50 mM KCl, 2.5 mM MgCl2, 0.5 µM of each primer, 0.15 mM dNTPs, 0.5 U of Platinum Taq DNA polymerase (Invitrogen) and 1 µL of DNA template. Thermocycler (Eppendorf Mastercycler pro S) conditions were: initial denaturation step of 94°C for 2 min; 35 cycles of 94°C for 30s, 60°C for 30s and 72°C for 1 min and final extension step of 72°C for 5 min. PCR products were purified with ExoSAP-IT (Affymetrix, Inc.). Sequencing was carried out at the University of Auckland DNA Sequencing Facility, New Zealand, using the BigDye Terminator version 3 sequencing kit and a capillary ABI3130XL (Applied Biosystems) DNA automated sequencer.

We used 14 microsatellite markers to genotype the populations. Eleven markers were developed by Jacob et al. (1995) from *Rattus norvegicus*, but have been used for *R. rattus* by many authors (e.g. Abdelkrim et al. 2010; Brouat et al. 2014; Pichlmueller and Russell 2018): D2Rat234, D11Mgh5, D5Rat83, D7Rat 13, D10Rat2, D12Rat76, D15Rat77, D16Rat81, D18Rat96, D19Mit2 and D20Rat46. Three other markers specifically designed for *R. rattus* characterized by (Loiseau et al., 2008) (Rr14, Rr17, Rr114) were used. Markers were chosen from different chromosomes to avoid physical linkage. Forward primers were labelled with fluorescent dyes: 6-FAM, HEX, VIC and PET (Applied Biosystems) (Supplementary Material 1).

The polymerase chain reactions (PCR) were performed as multiplex reactions in 10 µl volumes containing 1 µl of extracted DNA (>40ng DNA/ µl), 1 X QUIAGEN Multiplex PCR Master Mix and 0,2 µM of each primer used in the reaction. Loci were multiplexed in sets of two to seven. The thermocycler conditions were: initial heat activation of 95ºC for 15min, 30 cycles of 94º for 30s, 55ºC for 90s and 72ºC for 60s, and a final extension of 72ºC for 20min.

PCR products were diluted 10-fold, then 1 µl of the PCR solution was mixed with 0.4 µL of GeneScan™ 600 LIZ® (Thermo Fisher Scientific) and 10 µL of Hi-Di Formamide (Applied Biosystems), this mixture was submitted to heatshock treatment, 95ºC for 5min, 4ºC for 5min. Genotyping runs were performed on an ABI3130XL (Applied Biosystems) automated sequencer and analysed using GENEIOUS Prime 2019.0.4 (https://www.geneious.com) including the Microsatellite Analysis External Plugin version 1.4.6 (Biomatters Ltd.). To ensure scoring accuracy we re-genotyped 5% of samples.

Data analysis

For D-Loop, all the raw sequences were aligned in GENEIOUS Prime 2019.0.4 adjusted manually and trimmed to a common length of 520bp.

The software Micro-Checker version 2.2.3 Van Oosterhout et al. (2004) was used to test for the presence of null alleles, using Bonferroni correction and 3000 randomisations.

The number of alleles (NA), allelic richness (Ar), observed heterozygosity (HO), gene diversity within population (HS) were calculated with FSTAT 2.9.4 (Goudet 1995) for each population and all individual loci (Table 2). FIS and FST values were calculated according to (Weir and Cockerham, 1984). Pairwise FST values between populations (Table 3) were calculated and p-values were obtained with 95% confidence intervals, after 3,000 permutations. A test for departure from Hardy-Weinberg equilibrium was also carried out with FSTAT using 21,000 randomisations.

To approach the question of spatial genetic structure we use Bayesian clustering in software STRUCTURE 2.3.4 (Falush et al., 2003; Pritchard et al., 2000). The admixture model with correlated allele frequencies was run with 200,000 Markov chain Monte Carlo interactions for 10 runs, after a burning period of 1,000,000 and number of clusters ranging K = 1 to 4. The output file from STRUCTURE was used in STRUCTURE HARVESTER v0.6.94 (Earl and vonHoldt, 2012) to calculate ∆K values (the rate of change in the log probability of data between successive K values) as suggested by Evanno et al. (2005). Individual membership assignments estimated in STRUCTURE were aligned by

CLUMPP 1.1.2 (Jakobsson and Rosenberg, 2007) and DISTRUCT 1.1 (Rosenberg, 2004) was used to generate the bar plot.

We also perform principal components analysis (PCA) and use the saddlepoint approximation method described by (McMillan and Fewster, 2017) to visualize the genetic assignment of each sample. These analysis were performed in R 3.5.2 (R Core Team, 2017) with the package geneplot v0.1.0 (McMillan and Fewster, 2017).

Results

D-Loop

The sequencing of D-Loop region for the 58 individuals resulted in only 2 haplotypes that were geographically partitioned. Hap1 was found in all 35 individuals from the main island (Quixaba and Port populations) and Hap2 was found only in individuals from Rata Island. The haplotypes differ from each other by only one pair of base transition C↔T. Using the mitochondrial reference genome for *R. rattus* described for (Robins et al., 2008) (GenBank NC_012374) this transition is located at base position 15,757. The two haplotypes were deposited in GenBank (Accession numbers MN746385 and MN746386).

Microsatellite

A total of 58 individuals were caught and included in our study: 35 from the main island (25 from Quixaba village and 10 from the Port area) and 23 from Rata Island. The genotyping success was 99.7%. As expected, the population from Rata had lower diversity than the Quixaba population regarding number of alleles, allelic richness, gene diversity and observed heterozygosity (Table 1). Even the Port population, that had only 10 individuals genotyped, had higher allelic richness than the Rata population and the number of alleles was only slightly higher for Rata.

Table 1 Sampling location, number of sampled individuals (N_S), number of alleles (N_A), allelic richness (Ar), private alleles (PA), within population gene diversity (H_S), and observed heterozygosity (Ho; derived from H_S and F_{IS} values as $Ho=H_S-(F_{IS} \times HS)$), F_{IS} values (not significant, based on 42,000 randomisations).

Population	N_s	N_a	Ar	PA	H_s	H_o	F_{IS}
Quixaba	25	86	4.7	27	0.669	0.612	0.085
Port	10	60	4.3	12	0.594	0.600	-0.010
Rata	23	64	3.8	28	0.576	0.571	0.010

Micro-Checker detected excess of homozygotes for loci Rr14, Rr114 and D12 which can indicate presence of null alleles. Also, departure from Hardy-Weinberg equilibrium (HWE) was detected for those same loci (Table 2) Downstream analysis with and without these loci was performed, and since their presence did not show a significant effect, the following results are presented with all 14 loci included.

Table *2* Number of alleles (Na), allelic richness (Ar; based on min. sample size of 10 individuals), observed heterozygosity (Ho) and intrapopulation gene diversity (Hs) according to Nei (1987), F_{ST} and F_{IS} following Weir and Cockerham (1984) for each locus (***indicates significance $p < 0.001$, based on 21,000 randomisations).

Locus Name	Na	Ar	Ho	Hs	Fst	Fis
Rr14	10	5.502	0.388	0.47	0.569***	0.231***
D11	11	6.555	0.692	0.733	0.119***	0.068
Rr114	9	5.231	0.437	0.574	0.035***	0.327***
D7	9	6.683	0.699	0.665	0.251***	-0.039
D2	9	6.345	0.824	0.725	0.166***	-0.129
D18	13	8.34	0.725	0.767	0.171***	0.068
D5	9	6.223	0.759	0.681	0.225***	-0.14
D20	14	6.464	0.706	0.763	0.029***	0.055
D12	5	3.204	0.232	0.376	0.189***	0.411***
D16	7	5.788	0.708	0.634	0.312***	-0.109
D15	10	6.771	0.591	0.669	0.261***	0.062
D10	8	6.134	0.64	0.634	0.236***	0.024
D19	10	6.564	0.593	0.593	0.346***	0.013
Rr17	4	3.524	0.331	0.309	0.623***	-0.064
Average		5.952	0.595	0.614		

The pairwise F_{st} values suggests moderate genetic differentiation between the population from the main island Quixaba and Port ($F_{st} = 0.1073$, $p<0.01$), and suggests very great differentiation between those two populations and Rata ($F_{st} = 0.2956$ and $F_{st} = 0.3154$, $p<0.01$) (Table 3).

Table 3 Pairwise Fst values (** indicates significance *p* < 0.01 after 3,000 permutations)

	Port	**Rata**
Quixaba	0.1073**	0.2956**
Port		0.3154**
Rata Island		

The great differentiation between Rata and the two populations from the main island (Quixaba and Port) was confirmed by the Bayesian clustering analysis performed in STRUCTURE (Figure 3). This analysis, however, did not show differentiation between Quixaba and Port, indicating the most likely number of k = 2 (Supplementary Material 2).

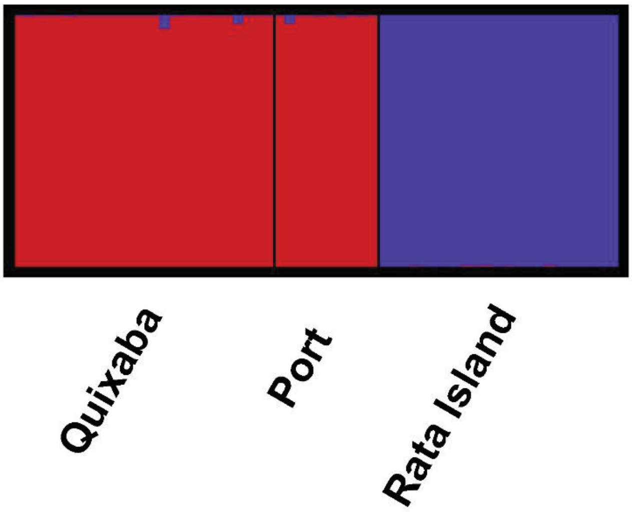

Figure. 3 STRUCTURE ancestry matrix without a priori assumption for assigning each sample in number of clusters. A most likely structure of k=2 across 10 replicates was indicated by STRUCTURE HARVESTER.

The genetic clustering in PCA (Figure 4A) grouped Quixaba and Port individuals while Rata’s individuals formed an isolated group. The saddlepoint approximation method used in GenePlot (Figure 4B) corroborated with PCA, STRUCTURE and Fst values indicating, a strong genetic differentiation between the populations of the two islands. The assignment of the Port population showed good fit to the Quixaba population for five individuals, meaning they are probably a subset of this population, while the other five individuals had poor fit to either population, and clustered with one individual from Quixaba that had poor fit to that population too. Overall, all analyses support genetic differentiation between the two island populations.

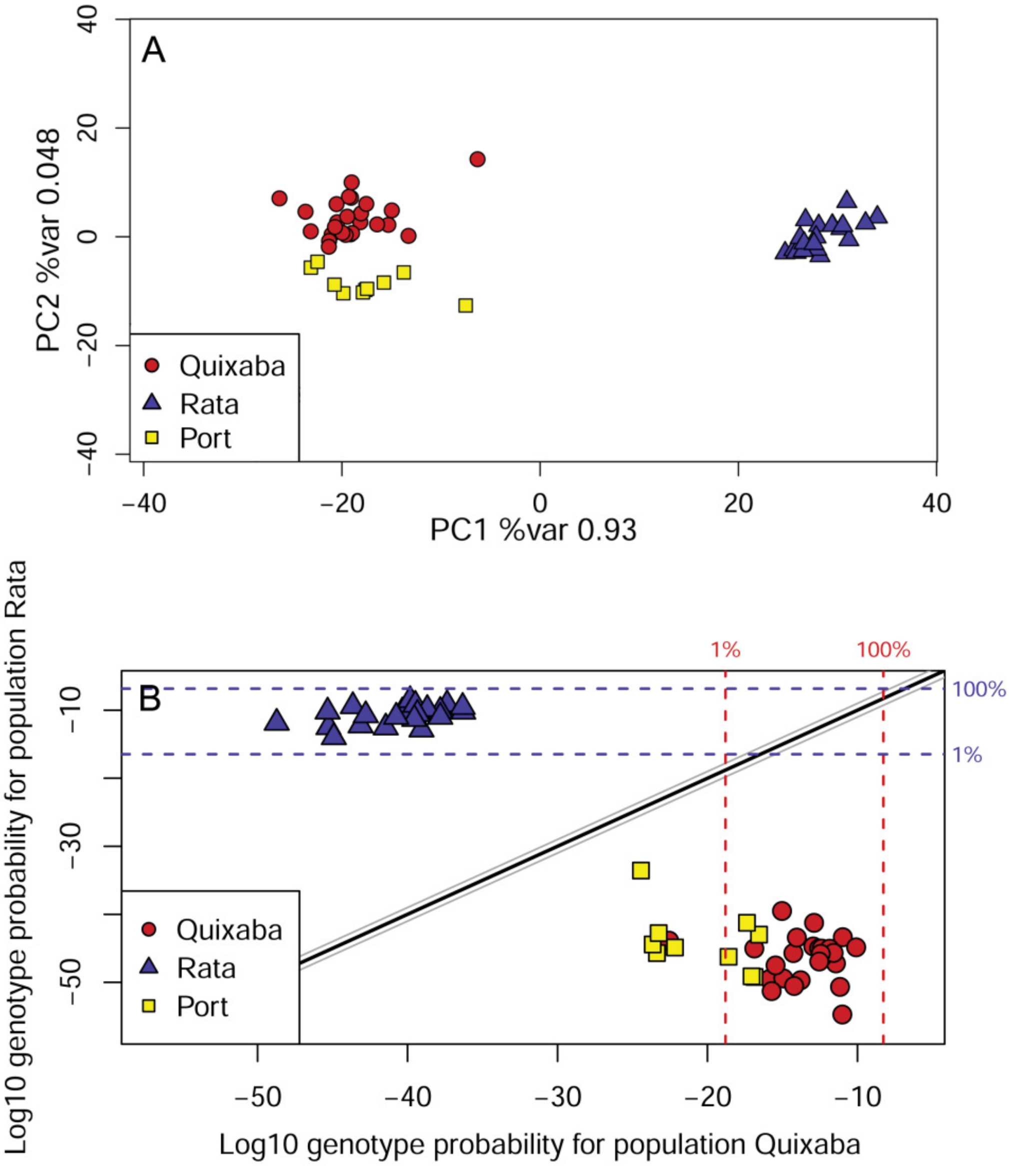

Figure. 4 Genetic assignments for sampled individuals. Principal Component Analysis (PCA), The first two principal components are shown and account for a total of 98 % variance explained (A). GenePlot using rats from Quixaba and Rata Island as reference and assignment for the Port individuals (B).

Discussion

All results indicate strong population differentiation between the main island and Rata, with no evidence of recent gene flow between both, indicating that eradication on Rata Island has a high probability to be sustained over time.

Invasion history

The presence of rats was officially reported in Fernando de Noronha by Branner in 1888, but their introduction most likely occurred in the 16th century (Carleton and Olson, 1999) when European ships, mostly from Portugal and France, started to berth in the archipelago (Silva, 2013b). Over the years, the location was visited by ships from other European countries which could have led to multiple invasion events on the main island but this is not supported by D-Loop data. Mitochondrial sequence data revealed only two haplotypes each presented on a different island, probably because of some priority effect that happens when an established population prevents new immigrant colonization (Fraser et al., 2014; Waters et al., 2013) or even due to genetic drift that might have caused loss of haplotypes and fixed the reported ones (Frankham et al., 2010). This pattern of a small number of introductions was also observed in larger scale in New Zealand, for *R. rattus* and *R. norvegicus* (Russell et al., 2019), and the Falkland Islands, for *R. norvegicus* (Hingston et al., 2016).

The difference between the two haplotypes was one base pair which suggests one could have derived from the other, but if this mutation was originated in the Noronha archipelago we would expect to see one of the islands presenting both haplotypes; the original and the mutated one, which did not occur. The alternative hypothesis is that each island was colonised in independent events of invasion that brought with them different haplotypes. Both haplotypes are recorded in other islands around the world, and were the two most common registered in New Zealand (Russell et al., 2019) (GenBank MH751483 and MH751484). Hap1 was also reported by Colangelo et al. (2015) as the most common haplotype in the Mediterranean basin. Another sequence similar to Hap1 was reported by Hingston et al. (2005) as “HaMI” (Haplotypes absent from Madagascar and India) and belonged to New York, Great Britain, France and French Polynesia locations. Unfortunately, the provided sequence for HaMI ended at position 15,769 while our fragment ended at 15,925 so we could not compare 156 bp to be sure that these are the exactly same haplotypes. Independent colonisations seem most likely, however, we are aware that our limited number of samples and coverage can have camouflaged a rare individual with a different haplotype on either island.

Rat eradication

Fernando de Noronha is located far from the coast and access to the archipelago is regulated, presenting a good potential for rat eradication. Still, even though most of the access to the archipelago happens by air on regular domestic aircrafts, there is heavy traffic of vessels and commodities between continental Brazil and the archipelago, promoting an infestation route for rodents. Noronha's satellite islands, however, are inaccessible to the general public, and visitation of researchers is only allowed escorted by the National Park staff and with their vessel. Moreover, these secondary islands are important breeding sites for most marine seabirds (Mancini et al., 2016) and endemic species as they are free of other predators such as cats and possibly the tegu lizard (Abrahão et al., 2019). If rat eradication could be achieved on these satellite islands, re-invasion that is human mediated (i.e. vessel hitchhiking) would be unlikely, and a surveillance program could keep these rat-free. Moreover, the knowledge gathered during such programs could inform eradication actions for the main inhabited island, since this is not a trivial task. Failure rate of rat eradication on tropical islands is 2 – 2.5 times higher than in islands located in high latitudes (Russell and Holmes 2015) and eradication in inhabited islands requires extra considerations (Oppel et al. 2011). The results indicated no recent gene flow occurring between islands, which leads us to be optimistic about eradication sustainability on Rata Island.

In the Galapagos archipelago Willows-Munro et al. (2016) found limited admixture (significant pairwise Fst values) even between islands belonging to the same proximate region, but in that scenario the closest islands did not shown Fst values as high as we found between Rata and the main island. In fact, islands further apart from each other like San Cristobal and Isabela (130 km) were more closely related than Rata Island and the main island here are. In

Noronha, despite the fact that the north-east island chain suggests a stepping stone model of gene flow we found very great differentiation between Rata Island and the two populations from the main island (Quixaba and Port) (Fst > 0.25) indicating presence of very low or no current gene flow between the islands, which is corroborated by the partition of mitochondrial haplotypes, found to be exclusive to each island tested. This lack of gene flow, although surprising, can be explained by the topography of the islands Rasa, Sela Ginete, Meio and Rata. All four have cliffs on their borders (Silva, 2013a) which can be a significant factor in limiting gene flow for *R. rattus*, even with its climbing skills, as reported by Fewster et al. 2011, besides there are three marine channels between the main island and Rata Island with strong water currents that could further make difficult the movement of rats between islands. Another possible explanation is that Sela Ginete is actually blocking gene flow between Rasa and Meio Islands because of its precipitous feature that can make it impracticable for rats to settle and disperse from there. In this case, it is possible that some gene flow occurs between Rasa and the main island and did occur prior to eradication between Meio and Rata. Unfortunately, we were not able to obtain samples from Sela Ginete due to its inaccessibility, if rats even exist there at all, and no samples were available from Meio Island prior to the rats being eradicated.

In contrast, the populations from Quixaba and Port, even being more distant from each other, present no barrier to dispersion besides distance and show only moderate differentiation according to Fst, suggesting presence of gene flow at some level. In the saddlepoint approximation method five Port individuals were shown as a subset of Quixaba and the other five individuals were assigned below the 1% line for the Quixaba population, meaning that even inside the same island there is some population structure. Besides, one individual from Quixaba was assigned below the 1% line for the Quixaba population as well, being grouped with the others from the Port and indicating probably an immigrant from the Port population.

Recommendations

Complete eradication in the archipelago is still not feasible due to legal restrictions and limited conservation funding Russell et al. (2018). Nevertheless, our findings suggested a low probability of a reinvasion in Rata occurring from the main island, notwithstanding possible priority effects blocking current dispersal. We were able to define at least two eradication units for Fernando de Noronha, which following a successful rat eradication on Meio Island (unpublished data) encourages the execution of a Rata Island eradication as proposed by Russell et al. (2018). A rat eradication associated with biosecurity measures to prevent new invasions by hitch hiking on boats could create a sanctuary to protect and restore breeding seabird colonies that are known to be in decline Mancini et al. (2016).

Data accessibility

Additional information can be found in the electronic supplementary material. The microsatellite data file is available in the Figshare digital repository and can be accessed at: https://doi.org/10.17608/k6.auckland.11307497.v1.

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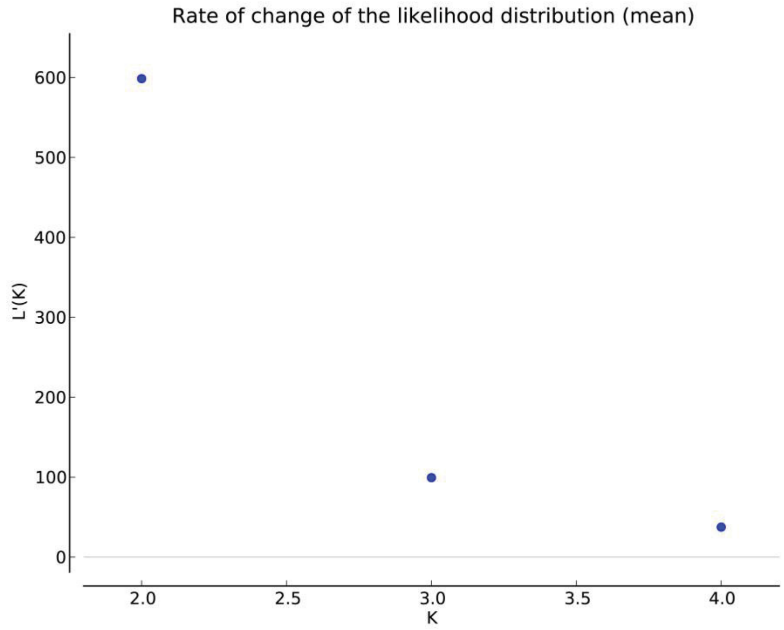
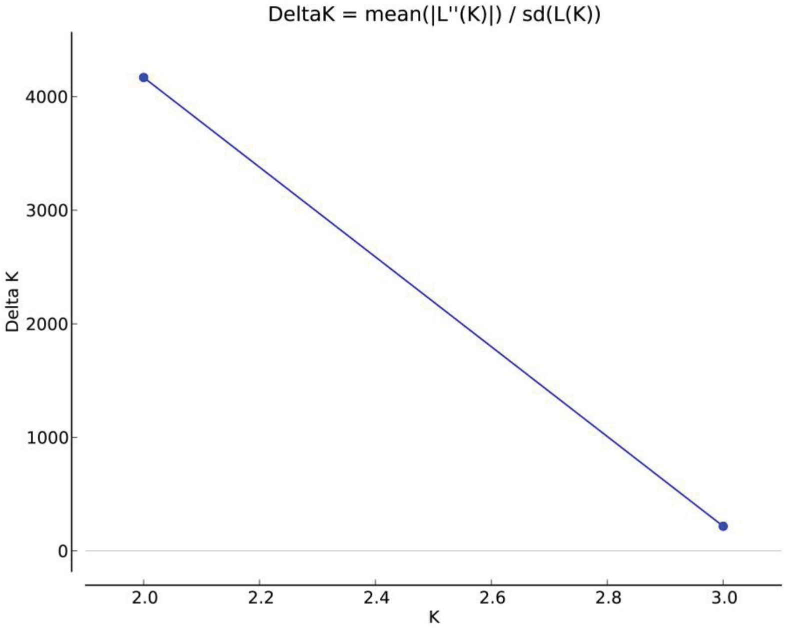
Supplementary Material 1

	Primer	Fluorescent Label	Sequence	Annealing Temperature
Multiplex Reaction 1	D2Rat234	HEX	F - ATATTCAAGCTGGCTTCCCC R - GTAGAGCAAGATGGGGTGGA	55ºC
	Rr14	6-FAM	F - CTGGCTGGGACAGTGGAG R - CGTCATCACTTCTCAGGACAG	55ºC
	D7Rat13	VIC	F - GACTTCTGCTACACGCCACA R - CAGCCCTAGAAGGAAATGCA	55ºC
	D5Rat83	PET	F - ACTTGGAAACAGGGAGATGG R - GGTCTTCAGGATGGCAATGT	55ºC
	D18Rat96	HEX	F - GCAGATCTCTCCTCCACAGC R - TGGACATCCTCAATGGACCT	55ºC
	D11Mgh5	6-FAM	F - CAGCTCTAATTCCAGAAAGGTTT R - GAATCGATTGACAGATGTCTGTG	55ºC
	Rr114	6-FAM	F - GCTGTGGCTAGAATCCAAGG R - ATGAGGCCTGTGGACGGTA	55ºC
Multiplex Reaction 2	D12Rat76	VIC	F - ATTGGCAATGCACTCATGTG R - TGCCTTTTAAAATGATGTGCA	55ºC
	D10Rat2	HEX	F - TGAGAACAGTCAAGGGGACA R - CCACTTAAGAGCCCAGCAAA	55ºC
	D20Rat46	6-FAM	F - GGCAAAACACCAATGCCTAT R - AAGTACTGAGTGGGCTGCGT	55ºC
	D16Rat81	VIC	F - GGCCCACATGTGCATGTATA R - GAGCCTTAGCACAGTGGCTT	55ºC
	D19Mit2	HEX	F - AAGGTTGGCAGTTTCCCAG R - ACCATTTATGTGCCCAGATG	55ºC
	Rr17	PET	F - CGTGTGGCATAGGTGAAGG R - TGCAGGAAACTGGTAGGACA	55ºC
	D15Rat77	VIC	F - ACAGAGGGAACCCATCACAG R - CATGTGGGGAAAGCATTACC	55ºC

Supplementary Material 2





6. CONSIDERAÇÕES FINAIS

Desde que o mundo começou a voltar sua atenção para o problema da Bioinvasão, este assunto têm sido negligenciado no Brasil. Mais especificamente, os estudos que têm como foco principal roedores invasores começaram tardiamente (2013), e por esse motivo, pouco se sabe sobre os ambientes que ocupam dentro do território nacional, como interagem com a fauna e flora nativas e quais áreas são prioritárias para programas de controle/erradicação. Esta tese procurou abranger cada uma destas abordagens tidas como objeto de estudo no mundo.

Dado que a ocupação de roedores invasores pode se dar de diferentes formas dependendo do ambiente em que se encontram, o capítulo I revelou que os ambientes preferenciais ocupados por *R. norvegicus* e *M. musculus* em uma cidade portuaria brasileira são aqueles mais afastados de grandes porções florestais. O ambiente florestal do bioma Mata Atlântica se mostrou, portanto, desfavorável à ocupação dessas espécies, revelando assim um serviço ecossistêmico antes subestimado. Dessa forma, expandir as florestas ao redor dos ambientes urbanos pode ser uma estratégia de contenção da expansão desses animais. Destaca-se também que para a espécie encontrada em maior abundância na cidade portuária (*R. norvegicus*), foi evidenciada forte preferência por áreas próximas à rodovia utilizada como acesso ao porto.

A partir dessa informação, o capítulo II avaliou a estrutura genética da população de *R. norvegicus* ao longo dessa rodovia e, ao contrário do que esperávamos, a dispersão mediada por humanos se mostrou mais frequente do que a dispersão natural, com áreas geograficamente mais distantes apresentando maior similaridade genética. Tal dispersão está muito provavelmente relacionada aos caminhões que transportam carga para dentro e para fora do porto. Tal informação serve de alerta para que medidas de biossegurança mais rigorosas sejam aplicadas nesse tipo de transporte, não só em Paranaguá, mas nos demais portos do Brasil.

Como último tema a ser abordado, os roedores invasores foram avaliados em ambiente insular. O capítulo III avaliou a factibilidade de uma proposta de erradicação para o arquipélago de Fernando de Noronha. Os dados genéticos indicaram ausência de fluxo gênico entre as ilhas avaliadas indicando assim que a Ilha Rata mais a nordeste do arquipélago se apresenta como ótima candidata a um programa de erradicação já que existe baixa probabilidade de recolonização proveniente da ilha principal.

A presente tese preencheu algumas das lacunas de conhecimento que dizem respeito a roedores invasores no Brasil, não somente em termos de biologia de populações, mas também com questões de manejo que são potencialmente aplicáveis a outros lugares do mundo. Além disso, a identificação de haplótipos (d-loop) no Parque Nacional de Fernando de Noronha alimenta o banco mundial de dados que auxiliam no monitoramento da distribuição destas espécies de roedores invasores, colaborando de forma ímpar para uma melhor compreensão da ocupação, expansão e domínio de áreas naturais por parte destes roedores invasores.

Embora algumas questões tenham sido respondidas, ainda há muito para ser esclarecido a respeito do assunto no Brasil. Dessa forma, mais estudos se fazem necessários para se entender questões a respeito das populações fonte dos ratos que chegam ao porto, estruturação da população de roedores dentro do porto, extensão e status de invasão nas ilhas na região da baia de Paranaguá, assim como nos quatro demais archipelágos presentes na costa brasileira.

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