Universidade de São Paulo
Faculdade de Saúde Pública

Biodiversidade de Culicidae e sua
interação com arboviroses e malária na
Mata Atlântica

Gabriel Zorello Laporta

Tese apresentada ao Programa de Pós-Graduação
em Saúde Pública para obtenção do título de
Doutor em Ciências.

Área de concentração: Epidemiologia
Orientadora: Profa. Dra. Maria Anice Mureb Sallum
Coorientador: Prof. Dr. Paulo Inácio de Knecht López
de Prado

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*à Ana e nossos filhos,
aos nossos pais, irmãos e sobrinhos,
às Famílias Laporta, Zorello e Galloro.*

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RESUMO

Laporta GZ. Biodiversidade de Culicidae e sua interação com arboviroses e malária na Mata Atlântica [tese de doutorado]. São Paulo (BR): Faculdade de Saúde Pública da USP; 2012.

Introdução - Interações complexas estão presentes entre a biodiversidade de mosquitos (Diptera, Culicidae) e as dinâmicas de transmissão de arbovírus e plasmódios que são agentes infecciosos que podem causar moléstias em humanos e outros animais. **Objetivos** - Aplicar método de distribuição potencial de habitats para mosquitos vetores de arbovírus e de plasmódios no Vale do Ribeira, sudeste do Estado de São Paulo, sub-região Serra do Mar da Mata Atlântica. Em escala local nessa região, relacionar a heterogeneidade espacial com a biodiversidade e esta com a dinâmica de transmissão de malária no Parque Estadual da Ilha do Cardoso. **Métodos** - Foram elaborados mapas de distribuição espacial dos vetores de arbovírus: *Aedes serratus*, *Aedes scapularis* e *Psorophora ferox*. Os mapas gerados para *Anopheles cruzii*, *Anopheles bellator* e *Anopheles marajoara* foram correlacionados com a distribuição espacial de malária. As correlações entre heterogeneidade espacial e biodiversidade de mosquitos foram estabelecidas com o emprego de modelos estatísticos de regressão. Foi elaborado modelo matemático para explicar o efeito da biodiversidade na transmissão de plasmódios. **Resultados** - As pessoas estão mais expostas às picadas de *Ae. serratus*, *Ae. scapularis* e *Ps. ferox* em áreas mais quentes e chuvosas. A correlação entre *An. marajoara* e o padrão espacial da malária foi positiva e significativa, enquanto que *An. cruzii* e *An. bellator* não foram importantes. Demonstrou-se que o aumento da heterogeneidade espacial está correlacionado, positivamente, com a biodiversidade de mosquitos. Níveis mais elevados de diversidade de mosquitos e de aves e mamíferos foram associados com risco menor de transmissão de plas-

módios. **Conclusões** - A modelagem de distribuição potencial de habitats é uma ferramenta para a vigilância de vetores de arbovírus. Recomenda-se maior atenção ao *An. marajoara* que poderia ser vetor secundário de plasmódios em áreas abertas, naturais e desmatadas, da Mata Atlântica. A diversidade de plantas aumenta a heterogeneidade espacial e, esta pode ter efeito positivo à biodiversidade de mosquitos. Maiores diversidades de mosquitos, aves e mamíferos poderiam diminuir o número de picadas infectivas de *An. cruzii*. Pesquisas futuras sobre a epidemiologia dessas doenças deveriam incluir os seguintes temas: mudanças climáticas e arboviroses, heterogeneidade espacial e mosquitos, e biodiversidade e malária.

Descritores: Ambiente e Saúde Pública; Epidemiologia; Mata Atlântica; Ecologia; Biodiversidade; Distribuição espacial; Modelos estatísticos; Malária-*vivax*; Arboviroses; Vetores.

ABSTRACT

Laporta GZ. Biodiversity of Culicidae and its interaction with arboviruses and malaria in the Atlantic Forest [Thesis]. São Paulo (BR): Faculdade de Saúde Pública da USP; 2012.

Introduction - Complex interactions are present between biodiversity of mosquitoes (Diptera, Culicidae) and the dynamics of vector-borne arboviruses and malaria-parasites, which are infectious agents that can cause diseases in humans and other animals. **Objectives** - to apply habitat-suitability modelling for arboviral and malarial mosquito vectors in Vale do Ribeira, southeastern São Paulo state, sub-region of Serra do Mar of Atlantic Forest. In a local scale of this region, to relate the spatial heterogeneity with biodiversity and the role of this with dynamics of malarial transmission in the Parque Estadual da Ilha do Cardoso. **Methods** - Potential distribution maps were generated for the vectors of arboviruses, such as *Aedes serratus*, *Aedes scapularis* and *Psorophora ferox*. Distribution maps generated for *Anopheles cruzii*, *Anopheles bellator* and *Anopheles marajoara* were correlated with spatial distribution of human malaria. Regression models were applied to correlate the spatial heterogeneity with biodiversity of mosquitoes. It was elaborated a mathematical model to explain the effect of biodiversity on the transmission of *Plasmodium*. **Results** - People are more exposed to bites of *Ae. serratus*, *Ae. scapularis* and *Ps. ferox* in warmer and wetter areas. Correlations between *An. marajoara* and spatial pattern of malaria were positive and significant, while *An. cruzii* and *An. bellator* were not important. Spatial heterogeneity was positively associated with biodiversity of mosquitoes. Higher levels of biodiversity of both mosquitoes and vertebrates (birds and mammals) was associated with low risk of *Plasmodium* transmission. **Conclusions** - Habitat-suitability modelling is a tool for the surveillance of vector-borne arbo-

viruses. It is recommended greater attention to *An. marajoara* which can be a potential secondary vector of *Plasmodium* parasites in natural or deforested open areas of the Atlantic Forest. Plant diversity could increase spatial heterogeneity which can be associated with higher mosquito diversity. Higher levels of mosquito, avian and mammalian diversities may decrease the number of infectious bites of *An. cruzii*. Future research on the epidemiology of malaria and arboviruses should include the following combinations of subjects: climate change and arboviruses, mosquito and spatial heterogeneity, and biodiversity and malaria.

Descriptors: Environment and Public Health; Epidemiology; Atlantic Forest; Ecology; Biodiversity; Spatial distribution; Statistical model; *vivax*-malaria; Arboviruses; Disease vectors.

PREÂMBULO

O texto dessa tese foi escrito em LaTeX que é um sistema de preparação de documentos¹. A estrutura textual da tese, ou seja, o *layout*, bem como a formatação do texto, p. ex., altura da linha e tamanho da fonte, foi automaticamente ajustada. O sumário, a lista de tabelas e de figuras reproduzem exatamente a estrutura textual da tese. O conteúdo nas listas de figuras e de tabelas é em inglês ou em português em acordo com a língua utilizada no texto. A abordagem LaTeX foi, portanto, empregada para manter o foco no conteúdo e na lógica das idéias, deixando a formatação por conta das macros, ou seja, *scripts* de programação disponíveis para esse sistema. Cabe ressaltar que este documento segue a formatação sugerida pelo guia de apresentação de teses da Faculdade de Saúde Pública da USP².

¹Lamport L. *L^AT_EX: A Document Preparation System*. Massachusetts: Addison Wesley; 1994.

²Cuenca AMB, Andrade MTD, Noronha DP, Ferraz MLEF. Guia de apresentação de teses. São Paulo: Biblioteca/CIR; 2008.

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4.13 Sensitivity analysis: if $h = 29$ then $R_0 < 1$. A, B: Decrease in abundance of non-vector mosquito species can increase risk of bromeliad malaria transmission ($R_0 > 1$) in indigenous Guarani Mbya settlement and Marujá fishing village, respectively; C, D: Decrease in abundance of non-host vertebrate species does not increase risk of bromeliad malaria transmission ($R_0 < 1$) in indigenous Guarani Mbya settlement and Marujá fishing village, respectively. D: Increase in abundance of non-host vertebrate species can increase risk of bromeliad malaria transmission ($R_0 > 1$) in Marujá fishing village, which is supported in the work by Saul (2003). 160

INTRODUÇÃO

Causadas por desmatamentos e demais atividades humanas, além de processos naturais, a fragmentação e degradação de habitats, perda de conectividade entre fragmentos, ausência de *stepping stones* e permeabilidade para a dispersão das espécies na paisagem podem causar o declínio da biodiversidade (Wilcox e Murphy, 1985; Hanski e Simberloff, 1997; Tokeshi, 1999; Metzger, 2000; Ricketts, 2001; Fahrig, 2003; Gibson et al., 2011). Quais seriam, do ponto de vista prático, as consequências desse declínio para a qualidade de vida dos seres humanos?

A biodiversidade mantém funções que fornecem bens e serviços ecossistêmicos. Um dos serviços é a regulação de doenças (Figura 1), significando que o impacto da biodiversidade na emergência/reemergência de doenças infecciosas é um novo foco em estudos de conservação (Ostfeld e Keesing, 2000; Begon, 2006; Keesing et al., 2010). Alguns autores observaram que, quando a biodiversidade diminui, o risco de doenças que afetam os corais (Cnidaria, Anthozoa) aumenta (Raymundo et al., 2009). Da mesma forma, o risco de esquistossomose (Johnson et al., 2009), febre do Nilo Ocidental (Swaddle e Calos, 2008), hantavíruses (Suzán et al., 2009), doença de Lyme (Keesing et al., 2009) e malária (Carlson et al., 2009) em seres humanos é maior quando a biodiversidade é negativamente impactada pelas atividades humanas. Portanto, a biodiversidade pode regular, indiretamente, a transmissão de agentes infecciosos aos humanos.

Figura 1: Biodiversidade, bens e serviços ambientais. (Autor e Fonte: CBD, 2006.)

Figura 2: Mapa das sub-regiões biogeográficas da Mata Atlântica baseadas em áreas de endemismo de aves, borboletas e primatas. (Autor: Ribeiro et al., 2009; Fonte: Silva e Casteleti, 2003.)

A Mata Atlântica é bioma de grande extensão distribuída em latitudes tropicais e sub-tropicais na América do Sul, contendo área de aproximadamente 16 milhões de hectares (SOS Mata Atlântica/INPE, 2008; Ribeiro et al., 2009; Figura 2). O bioma é bastante heterogêneo devido às complexidades geológicas, topográficas e climáticas (Ab'Saber, 1977; Suguio e Martin, 1978; Oliveira-Filho e Fontes, 2000; Alves et al., 2010) que resultaram em diversos tipos de ecossistemas com elevado grau de riqueza e endemismo de espécies (Müller et al., 1972; Brown e Ab'Saber, 1979; Morellato e Haddad, 2000; Galindo-Leal e Câmara, 2003; Hil-

lebrand, 2004; Lewinsohn e Prado, 2005; Tabarelli et al., 2005; Fitzpatrick et al., 2009; Galetti et al., 2009). Entretanto, os remanescentes de Mata Atlântica são geralmente pequenos e sem conectividade (Ribeiro et al., 2009, 2011), sendo principalmente formados por florestas secundárias em estágios iniciais e intermediários de regeneração (Viana et al., 1997; Metzger et al., 2009). Considerando os altos níveis de endemismo, a elevada riqueza em espécies e as altas taxas de perda de habitat, a comunidade científica brasileira e internacional reconhece que a conservação e restauração da diversidade biológica nesse bioma são prioritárias (Brown e Brown, 1992; Myers et al., 2000; Laurance, 2009; Joly et al., 2010).

O sistema Serra do Mar (Figura 2) inclui-se na sub-região biogeográfica homônima, constituindo corredor de dispersão de vertebrados e invertebrados no domínio da Mata Atlântica, de acordo com Silva e Casteleti (2003) e Ribeiro et al. (2009). Na porção central do corredor sul-sudeste da Serra do Mar, situa-se região preservada e biodiversa (Ribeiro et al., 2009, 2011) denominada Vale do Ribeira, Estado de São Paulo (Figura 3C). Em relação ao relevo, o Vale do Ribeira possui paredão montanhoso (Encosta de Serra) e terreno que se estende até a orla marítima (Planície Litorânea). A Encosta se orienta no sentido nordeste-sudoeste, aproximando-se da costa, e assim emoldura toda a extensão da Planície, a qual constitui uma das maiores formações sedimentares em zona tropical. A região apresenta clima subtropical e tropical úmido, apresentando fitogeografia predominantemente florestal cuja fisionomia, de aspecto denso e de elevado grau de heterogeneidade, é representada por indivíduos arbóreos de porte elevado (30 a 40 m de altura). Como exceção, deve-se considerar a faixa costeira com vegetação litorânea representada por pioneiras de dunas, mangues e cobertura arbórea-arbustiva conhecida como “restinga” (Figura 3C) onde geralmente se situam os núcleos urbanos locais (Forattini et al., 1978a). Portanto, o mosaico de ocupação humana com vegetação natural pode representar risco de emergência/reemergência de doenças associadas a mosquitos (Figura 4).

Figura 3: A: América do Sul e Brasil; B: Remanescentes naturais no Estado de São Paulo; C: Vale do Ribeira. A quadricula (C) é 23J, com datum horizontal WGS84 e datum vertical Imbituba. (Fonte: SOS Mata Atlântica/INPE, 2008.)

Figura 4: Distribuição do risco relativo de ocorrência de doenças infecciosas emergentes causadas por arbovírus, protozoários e helmintos transmitidos por vetores (menores e maiores riscos representados, respectivamente, pelas cores verdes e vermelhas). No continente sul-americano, a seta indica a posição do Estado de São Paulo no mapa. (Autor e Fonte: Jones et al., 2008.)

MALÁRIA E VETORES DE PLASMÓDIOS

O número de mortes por malária diminuiu nas últimas décadas, embora aproximadamente meio milhão de casos são esperados a cada ano, principalmente na região Amazônica (Oliveira-Ferreira et al., 2010).

A malária foi problema de saúde pública (i.e., risco elevado de transmissão) nas regiões de Mata Atlântica do Estado de São Paulo antes da década de 1970. Como resultado das campanhas de erradicação realizadas durante a década de 1960, a incidência da malária diminuiu de aproximadamente 40 mil casos anuais em 1940 para 5.000 casos em 1970 e 70 casos em 1982 (Deane, 1988). No Vale do Ribeira, a média de casos autóctones notificados esperados por ano é 19 (IC95% = 0 - 39) (Figura 5). A incidência anual da doença (número de casos-ano dividido por 100.000 habitantes) variou entre 0.05 e 0.25 no período que compreende de 1980 a 2007. Esses casos são representados por infecções sintomáticas por *Plasmodium vivax*, com febre e calafrio sendo os sintomas mais comuns (Couto et al., 2010). Entretanto, infecções assintomáticas em habitantes dessa região foram detectadas em bancos de sangue (Lima et al., 2011). A incidência das infecções assintomáticas seria, significativamente, maior do que as sintomáticas para as populações da Mata Atlântica do Estado de São Paulo (1-2%, Bydlowski, comunicação pessoal).

Os mosquitos, conhecidos vetores de plasmódios na Mata Atlântica, são aqueles do subgênero *Kerteszia*, por exemplo, *Anopheles cruzii*, *Anopheles bellator* e *Anopheles homunculus* nos estados de Santa Catarina, Paraná e São Paulo (Lutz, 1903; Coutinho et al., 1943; Rachou, 1946; Coutinho, 1947; Rachou e Ferreira, 1947; Pinotti et al., 1947; Forattini et al., 1999; Marrelli et al., 2007; Marques et al., 2012). Cerutti et al. (2007) sugeriram que o *An. cruzii* poderia estar envolvido na dinâmica de transmissão da malária em regiões serranas no Espírito Santo. *Anopheles marajoara* e *Anopheles aquasalis*, duas espécies do

Figura 5: Número total de casos de malária no sudeste do estado de São Paulo, Mata Atlântica, de 1980 a 2010. (Fonte: Couto et al., 2010; CVE, 2011.)

subgênero *Nyssorhynchus*, são consideradas vetores locais ou regionais, sendo a primeira no sudeste e a segunda no nordeste e norte da costa Atlântica do Brasil (Prado e Carvalho, 1929; Schiavi, 1945; Coutinho, 1947; Correa et al., 1950; Rachou, 1958; Deane, 1986; Zimmerman, 1992; Rosa-Freitas et al., 1998; Meneguzzi et al., 2009; Laporta et al., 2011a). Ainda considerando-se o mesmo bioma, vale assinalar que o mosquito *Anopheles darlingi*, vetor primário na Região Amazônica, pode também participar da dinâmica de malária na Mata Atlântica, pois o limite de distribuição geográfica do inseto atinge o sudoeste do Estado do Paraná (Coutinho, 1947; Deane, 1986; Zimmerman, 1992; Rosa-Freitas et al., 1998; Pedro e Sallum, 2009).

ARBOVIROSES E VETORES DE ARBOVÍRUS

Arbovírus é microparasito que circula entre vertebrados e invertebrados, ocasionando a formação de ciclos de transmissão os quais poderiam emergir em áreas antrópicas.

O Vale do Ribeira proporciona condições ecológicas para a emergência dos arbovírus Rocio (ROCV), febre amarela (YFV), encefalite equina venezuelana (VEEV), Saint Louis (SLEV) e encefalite equina do leste (EEEV), que podem causar quadros de encefalites graves em humanos (Lopes et al., 1979; Calisher et al., 1982, 1983; Iversson, 1988; Bedin, 2007; Figueiredo, 2007). Ocorreram 1.100 casos humanos de encefalite por ROCV, com letalidade oscilando entre 10 e 15%, nessa região, entre 1975 e 1983 (Iversson, 1988). Desde 1999, a área de incidência de febre amarela, antes restrita à região amazônica, começou a expandir e incluir outras regiões do Brasil (Vasconcelos, 2003) como, por exemplo, o interior dos estados de São Paulo (Souza et al. 2010) e Rio Grande do Sul (Cardoso et al. 2010), aumentando o risco de introdução do YFV na Mata Atlântica. Em pesquisa sobre outras arboviroses, que envolveu a análise do perfil de anticorpos IgG de 182 residentes da Estação Ecológica de Juréia-Itatins, Vale do Ribeira, a prevalência para VEEV foi 19,2% enquanto para SLEV foi 7,1% e para EEEV, 2.1% (Romano-Lieber e Iversson, 2000).

As dinâmicas de transmissão dessas arboviroses podem envolver interações entre mosquitos e reservatórios (animais silvestres), sendo os humanos hospedeiros acidentais (Lopes et al., 1978a,b; Lopes et al., 1981; Mitchell e Forattini, 1984; Mitchell et al., 1986; Forattini e Massad, 1998; Figueiredo, 2007). *Aedes scapularis* e *Psorophora ferox* são os prováveis vetores de ROCV, sendo o primeiro na área rural e o segundo na mata (Lopes et al, 1981; Mitchell et al., 1986; Forattini et al, 1995a). Os principais vetores de YFV são mosquitos do gênero *Haemagogus* e *Sabethes* (Vasconcelos, 2003). No entanto, vale assinalar que ou-

tras espécies podem participar da transmissão do agente, por exemplo, o YFV foi isolado de *Aedes serratus* no Rio Grande do Sul, em 2008 (Cardoso et al. 2010). Essa espécie é muito abundante no Vale do Ribeira, onde poderia ser potencial vetor de YFV (Forattini et al., 1995a; Laporta et al., 2011b). Os VEEV, SLEV e EEEV circulam “silenciosamente” na Mata Atlântica (Lopes et al., 1979; Calisher et al., 1982; Ferreira et al., 1994) porque a transmissão é restrita aos ciclos enzoóticos em que o vírus circula entre reservatórios e mosquitos associados à mata. Mosquitos do subgênero *Melanoconion* de *Culex* são vetores de VEEV (Weaver e Reisen, 2010) e são abundantes no Vale do Ribeira (Forattini et al., 1995b). *Culex coronator* e *Culex declarator* estão presentes em remanescentes dessa região (Forattini et al., 1995c), onde poderiam desempenhar papel no ciclo enzoótico de SLEV (Vasconcelos et al., 1998). No Brasil, *Culex pedroi* é vetor de EEEV (Kondig et al., 2007), sendo encontrado nos remanescentes de vegetação do Vale do Ribeira (Forattini et al., 1995b). Adicionalmente, *Mansonia titillans*, *Culex nigripalpus* e *Coquillettidia venezuelensis* ocorrem em alta abundância nas áreas rurais dessa região (Forattini et al., 1978a,b; Forattini et al. 1995c; Forattini e Massad, 1998), onde poderiam transmitir VEEV, SLEV e EEEV aos humanos, respectivamente.

HABITATS DE CULICIDAE

Na Mata Atlântica do Vale do Ribeira, a diversidade de mosquitos é elevada, com espécies que apresentam padrões diversos de utilização de recursos providos pelos múltiplos habitats. Naquela região, foram encontradas aproximadamente 200 espécies, identificadas ou descritas (Forattini et al., 1978a,b, 1981, 1986, 1993a,b,c, 1995a,b,c; Sallum e Forattini, 1996; Forattini et al., 1998a; Sallum et al., 2009; Nagaki et al., 2011). Os mosquitos utilizam vários tipos de

habitats larvais que são influenciados, diretamente, pela presença e composição das plantas, podendo ser observados dois grupos principais: mosquitos de habitat larval no solo e mosquitos associados às fitotelmatas, i.e., coleções hídricas que se acumulam em partes de vegetais (Shannon, 1931; Bates, 1949; Forattini, 1962; Forattini, 1965a,b; Clements, 1999; Juliano, 2009). As especializações presentes nos aparelhos bucais das larvas (Harbach, 1977; Merritt e Cummins, 1984; Laird, 1988; Merritt et al., 1992; Forattini 1996) refletem o tipo de alimento e como o mesmo é obtido por cada espécie no habitat larval. Nesse contexto, os mosquitos podem ocupar grande variedade de habitats representados por coleções de água tanto no solo como em fitotelmatas.

Por exemplo, imaturos de *Psorophora* e do subgênero *Ochlerotatus* de *Aedes* se desenvolvem em águas acumuladas na forma de poças temporárias. Imaturos dos subgêneros *Culex* e *Melanoconion* de *Culex* são frequentes em habitats larvais no solo com alta concentração de matéria orgânica, natural ou induzida pelo homem (Forattini, 1965a). Larvas e pupas de *Mansonia* e *Coquillettidia* retiram o ar acumulado no parênquima aerífero da vegetação aquática presente em habitats permanentes, no solo (Forattini, 1965b). As larvas do subgênero *Nyssorhynchus* de *Anopheles* se desenvolvem em coleções hídricas presentes no solo em ambientes rurais (Forattini et al., 1993a,b). Das espécies que utilizam fitotelmatas, aquelas dos subgêneros *Kerteszia* de *Anopheles*, *Microculex* de *Culex* e *Phomyia* de *Wyeomyia* são frequentemente encontradas em bromélias (Forattini et al., 1986; Marques et al., 2012). Espécies da tribo Sabethini, do gênero *Haemagogus* e do subgênero *Howardina* de *Aedes* são encontradas em fitotelmatas em remanescentes de vegetação (Forattini, 1965a; Forattini, 1965b). *Aedes albopictus* foi introduzido no Brasil na década de 1980 (Forattini, 1986) e, atualmente, está adaptado às condições nativas, sendo encontrado em bromélias no ambiente urbano (Forattini et al., 1998b) e buracos de árvores, no solo, na zona rural (Forattini et al., 1998c).

ALTERAÇÕES AMBIENTAIS E EMERGÊNCIA DE VETORES

Mudanças climáticas, modificações no uso da terra e super-exploração dos recursos ecossistêmicos têm efeito sobre a riqueza, abundância e dominância de espécies de mosquitos (Dorvillé, 1996; Forattini e Massad, 1998; Massad e Forattini, 1998). As plantas que formam a estrutura das florestas podem estar envolvidas, direta ou indiretamente, na disponibilidade de alimentos para as formas iniciais de vida de espécies de mosquitos. Forattini et al. (1986) observaram alta riqueza de espécies de solo e de fitotelmatas, além da dominância de *An. cruzii*, na mata de encosta em Cananéia, Vale do Ribeira. As espécies que dependem de fitotelmatas podem ser eliminadas de locais que sofreram alterações no ecossistema natural ou, as mesmas dispersam para os remanescentes florestais adjacentes à área impactada (Forattini et al., 1993c). Das espécies de fitotelmatas, aquelas do subgênero *Kerteszia* e *Aedes albopictus*, que é alóctone (Forattini, 1986), podem colonizar bromélias no ambiente antrópico (Forattini et al., 1998b; Forattini et al., 2000). Nesse ambiente, a riqueza de espécies é em geral menor, mas abundância total pode ser elevada (Ueno, 2000). Espécies de *Mansonia* e *Coquillettidia* e dos subgêneros *Nyssorhynchus* de *Anopheles*, *Ochlerotatus* de *Aedes* e *Culex* e *Melanoconion* de *Culex* podem ser abundantes e dominantes no ambiente rural (Forattini et al., 1978a,b, 1981, Forattini et al., 1993a,b, 1996a,b,c; Forattini e Massad, 1998).

ALTERAÇÕES AMBIENTAIS E EMERGÊNCIA DE DOENÇAS ASSOCIADAS A VETORES

O desmatamento seguido do aumento da população humana podem causar risco elevado de transmissão de plasmódios e arbovírus (Oliva e Sallum, 1996; Fo-

rattini, 1998; Forattini e Massad, 1998; Laporta et al., 2011a). O desmatamento pode criar condições ecológicas e ambientais associadas a maior abundância de espécies do subgênero *Nyssorhynchus* de *Anopheles* (Forattini et al., 1993b), e de espécies vetoras de arbovírus, dos gêneros *Mansonia* e *Coquillettidia* (Forattini et al., 1978a,b), dos subgêneros *Ochlerotatus* de *Aedes* (Forattini et al., 1995a) e *Culex* e *Melanoconion* de *Culex* (Foratini et al., 1995b,c) e *Aedes albopictus* (Fernández et al., 2003). A aglomeração humana eleva a proporção de hospedeiros suscetíveis, podendo aumentar a probabilidade de introdução de novos parasitos na população (Oliva e Sallum, 1996).

Como consequência de alterações no ambiente natural da Mata Atlântica, ocasionadas por atividades humanas, vale assinalar a ocorrência de 1.100 casos de encefalite pelo vírus Rocio, Vale do Ribeira, nas décadas de 1970 e 1980 (Lopes et al., 1978a; Iversson, 1988). Esse vírus foi detectado em diversas espécies de aves e mamíferos da Mata Atlântica, das quais ao menos uma, tico-tico (*Zonotrichia capensis*), poderia ser reservatório capaz de infectar mosquitos *Psorophora ferox* em áreas de mata (Lopes et al., 1978b, 1981), representando assim o foco silvestre. Ao remover parte da cobertura florestal para a construção de moradias, houve aumento de fontes sanguíneas para os mosquitos e do número de possíveis criadouros de mosquitos. Dessa maneira as condições se tornaram favoráveis para o aumento da população de outro vetor, *Aedes scapularis* (Forattini et al., 1978a,b, 1981). Este mosquito participou da dinâmica de transmissão do ROCV no ambiente extra domiciliar, o que tem suporte com os trabalhos de campo sobre a ecologia do mosquito (Forattini et al., 1981) e estudos de competência vetora conduzidos em laboratório (Mitchell e Forattini, 1984; Mitchell et al., 1986).

MODELOS MATEMÁTICOS

A existência do risco de transmissão de agentes infecciosos por mosquitos no Vale do Ribeira e a inerente inviabilidade ética de fazer estudos epidemiológicos experimentais motivaram pesquisas em que a dinâmica de transmissão é descrita por meio de modelos matemáticos (Oliva e Sallum, 1996; Massad e Forattini, 1998). Utilizando generalizações dos modelos de Ross (Lotka, 1923), Oliva e Sallum (1996) elaboraram modelo matemático para descrever a dinâmica de malária em uma estação experimental de arroz irrigado, município de Registro, Vale do Ribeira. Esse modelo previu que o aumento do risco de transmissão de parasitos da malária está correlacionado com a abundância dos potenciais vetores do complexo *Anopheles albitarsis* (Forattini et al., 1993a,b,c), aumento de hospedeiros e proporção de infectados na população de suscetíveis (Oliva e Sallum, 1996). Massad e Forattini (1998), utilizando o R_0 , i.e., número de infecções secundárias na população de suscetíveis produzidas por uma única pessoa infectada durante o período de infecciosidade da mesma, demonstraram que o aumento da temperatura global (i.e., mudanças climáticas) diminui o tempo de desenvolvimento larval e aumenta a taxa de picadas de *Anopheles*, indicando aumento no risco de ocorrência de malária (Massad e Forattini, 1998).

RELAÇÃO DA BIODIVERSIDADE COM DOENÇAS ASSOCIADAS A MOSQUITOS

O risco de transmissão de agentes infecciosos por mosquitos é presente, mas qual seria a relação desse risco com a biodiversidade pertencente às paisagens conservadas no Vale do Ribeira? Segundo Ribeiro et al. (2009, 2011), o Vale do Ribeira representa uma das porções mais bem preservadas da Mata Atlântica.

A biodiversidade poderia regular a transmissão de plasmódios e arbovírus aos humanos nessa região? Keesing et al. (2010) sugerem ambos efeitos, positivos e negativos, da biodiversidade no risco de doenças infecciosas. Quando a doença está bem estabelecida no local a biodiversidade diminui o risco (Keesing et al., 2010). Por exemplo, a abundância de *Anopheles gambiae* s.s. pode diminuir devido à maior diversidade de predadores e competidores de larvas do mosquito na África sub-Sahariana (Carlson et al., 2009; Kirby e Lindsay, 2009). Entretanto, altos níveis de biodiversidade podem prover fonte potencial de patógenos emergentes. Por exemplo, o surgimento do vírus do Nilo Ocidental em humanos na África na década de 1950 e a sua expansão geográfica para a América do Norte causou aproximadamente 20.000 casos de encefalite humana notificados nos E.U.A., de 1999 a 2006 (Kramer et al., 2007).

JUSTIFICATIVA

A emergência ou a reemergência de arbovírus silvestres pode causar casos graves de encefalite humana no sudeste da Mata Atlântica (Lopes et al., 1978a,b, 1979, 1981; Forattini et al., 1978a,b, 1981; Calisher et al., 1982, 1983; Mitchell e Forattini, 1984; Mitchell et al., 1986; Iversson, 1988; Bedin, 2007; Figueiredo, 2007). Em 1975, a expansão do foco silvestre de transmissão de ROCV em direção às áreas antrópicas se tornou mais provável devido ao comportamento da fêmea de *Ae. scapularis* que percola paisagens desmatadas para obter sangue de hospedeiros (Forattini et al., 1995a). Esses focos antrópicos continuaram a se expandir para localidades vizinhas em progressão temporal formando uma onda epidêmica (Figura 6). A doença continuou a ser notificada até 1983, e em seguida recrudescceu (Iversson, 1988).

A epidemia de encefalite por ROCV mostra a importância de se aplicar a

vigilância de forma preventiva. Por exemplo, mapas de vetores de arbovírus em escalas adequadas às ações de vigilância podem ser produzidos, utilizando abordagens como a modelagem da distribuição potencial de habitats (Diuk-Wasser et al., 2006; Foley et al., 2007; Ayala et al., 2009; Hay et al., 2010; Sinka et al., 2010). Com essa abordagem é possível superar os desafios ao monitoramento como a escassez de dados de campo e a magnitude territorial do Brasil.

Figura 6: Incidência de encefalite por arbovírus Rocio no Vale do Ribeira e litoral sul, sudeste do estado de São Paulo, sub-região Serra do Mar da Mata Atlântica. (Autor e Fonte: Iversson, 1988.)

A dinâmica de transmissão de malária é complexa porque depende de variáveis ambientais, ecológicas e sociais (Tadei et al., 1998; Castro et al. 2006; Meyrowitsch et al., 2011). A ocupação humana nos ambientes naturais pode causar a remoção de florestas e formação de áreas para agricultura, exploração de madeira e diversas atividades econômicas. Particularmente as florestas tropicais estão ameaçadas por essa ocupação, o que pode afetar negativamente a estrutura

biológica desses ecossistemas, diminuindo a riqueza de espécies (Tews et al., 2004; Díaz et al., 2005; Pardini et al., 2005; Gibson et al., 2011). Castro et al. (2006) demonstraram que os estágios iniciais dos assentamentos rurais na Amazônia em fronteira com as matas são dominados pelos riscos associados a transmissão de parasitos da malária em Machadinho, Estado de Rondônia. Segundo Ostfeld et al. (2005) e Reisen et al. (2010), as modificações, naturais ou induzidas pelo homem, na estrutura e na heterogeneidade da paisagem podem criar condições ecológicas e microclimáticas que favorecem algumas espécies de insetos vetores e os parasitos, por exemplo, os plasmódios, que são transmitidos por eles. Vittor et al. (2006) mostrou que o desmatamento facilitou o contato entre *An. darlingi* e humanos na Amazônia peruana, aumentando a incidência de malária naquela região. Olson et al. (2010) mostrou que 4.3% de mudanças na paisagem natural, provocadas por desmatamento, foi correlacionado com aumento de 48% na incidência de malária no município de Mâncio Lima, Estado do Acre. Da mesma forma, no Vale do Ribeira, as condições ecológicas que favorecem a presença de habitats para *An. marajoara*, combinado com a migração de indivíduos infectados com plasmódios humanos, poderiam mudar o status atual da malária de baixa endemicidade para situação de maior risco. Salienta-se, entretanto, que o elevado risco de transmissão de plasmódios ocorre quando a espécie é dominante na comunidade de mosquitos, o que está em função da proporção de mata na paisagem (Figura 7).

A presença de casos de malária no Vale do Ribeira parece estar associada a ciclos complexos que envolvem diversas espécies de *Plasmodium* e hospedeiros primatas não humanos. A circulação de *P. malariae*, *P. falciparum*, *P. vivax* e variantes (Curado et al., 2006) ocorre em ciclos não totalmente revelados que proporcionam a presença de infecções inaparentes que, raramente, são detectadas pelos métodos diagnósticos de rotina (Ministério da Saúde, 2005) e assim permanecem imperceptíveis à vigilância (Müller et al., 2007). Adicionalmente, macacos

Figura 7: Curvas hipotéticas (i.e., ajustadas a partir do conhecimento teórico) de abundância de três dos principais vetores de plasmódios do Brasil segundo o tipo de paisagem: mata, zona rural e cidade.

Alouatta podem ser reservatórios de plasmódios que poderiam infectar humanos (Figura 8A), acidentalmente. Finalmente, diversos vetores poderiam participar da dinâmica de transmissão da malária: *An. cruzii* em paisagem natural (Figura 8A), *An. cruzii*, *An. bellator* e *An. homunculus* em paisagem fragmentada (Figura 8B), *Anopheles aquasalis* e *Anopheles marajoara* em paisagem modificada (Figura 8C), gerando a possibilidade da participação concomitante dos ciclos silvestre e rural de transmissão de plasmódios e, assim, da exacerbação da malária no Vale do Ribeira.

Figura 8: Dinâmica de transmissão de parasitos da malária em três tipos de paisagem na sub-região Serra do Mar da Mata Atlântica. A: Dinâmica de transmissão silvestre em que bugios (*Alouatta*) podem ser reservatório de plasmódios humanos, os quais são transmitidos por *An. cruzii* (Deane et al. 1965, 1966; Duarte et al., 2008); B: Dinâmica de transmissão silvestre com a presença humana, na qual residência próxima da vegetação pode aumentar o risco de humanos expostos às picadas *Plasmodium*-infectantes de *An. cruzii* (Lutz, 1903; Rachou, 1946), *An. bellator* (Coutinho et al., 1943; Rachou e Ferreira, 1947) e *An. homunculus* (Pinotti et al., 1947); C) Dinâmica de transmissão em paisagem naturalmente aberta (Correa et al., 1950) ou desmatada (Laporta et al., 2011a), na qual os humanos podem estar expostos às picadas *Plasmodium*-infectantes de *An. aquasalis* (Prado e Carvalho, 1929) e *An. marajoara* (Schiavi, 1945). A autoria do desenho do anofelino na posição de repouso e das fotografias em branco-e-preto é do Prof. O.P. Forattini.

HIPÓTESES

A região do Vale do Ribeira é a maior e mais bem preservada porção remanescente da Mata Atlântica (Silva e Casteleti, 2003; Ribeiro et al., 2009) e foi considerada propícia à emergência/reemergência de doenças nas quais os parasitos são transmitidos por vetores (Figura 4, segundo Jones et al., 2008). Portanto, a hipótese nula desta tese é:

- H_0 : a biodiversidade da Mata Atlântica **não** interfere na emergência de doenças infecciosas associadas a mosquitos.

Entretanto, a biodiversidade pode interagir com a dinâmica de doenças infecciosas, podendo ter um papel regulador e, assim, diminuindo o risco de exposição dos humanos aos parasitos transmitidos por vetores (Keesing et al., 2010). Assumindo essa afirmação como verdadeira, a hipótese alternativa é:

- H_A : a biodiversidade da Mata Atlântica *interfere*, positiva ou negativamente, na dinâmica de transmissão de malária e arboviroses.

OBJETIVO GERAL

Caracterizar a associação entre biodiversidade de Culicidae e ocorrência de arboviroses e malária no sudeste da Mata Atlântica (Vale do Ribeira).

OBJETIVOS ESPECÍFICOS

- Caracterizar a distribuição potencial de habitats para *Ae. scapularis*, *Ae. serratus* e *Ps. ferox*;

- Verificar a utilidade de modelos de distribuição de habitats, como ferramenta para a vigilância de arboviroses;
- Caracterizar a distribuição potencial de habitats para *An. cruzii*, *An. bellator* e *An. marajoara*;
- Verificar se existe associação entre a presença de habitats potenciais para *An. cruzii*, *An. bellator* e *An. marajoara* e a distribuição de casos autóctones de malária;
- Analisar o efeito da disponibilidade e heterogeneidade de habitats na riqueza, abundância total e dominância de espécies de mosquitos;
- Verificar se a disponibilidade e heterogeneidade de habitats podem regular a emergência de vetores;
- Analisar os efeitos da abundância de mosquitos não vetores e de mamíferos/aves na dinâmica da transmissão de malária;
- Elaborar modelo matemático da dinâmica de transmissão de malária associada a mosquitos vetores *Anopheles (Kerteszia) cruzii*.

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Capítulo 1

Spatial distribution of arboviral mosquito vectors in the Vale do Ribeira

Nesse capítulo, que foi publicado nos Cadernos de Saúde Pública¹, é demonstrada a aplicação de um método estatístico para a inferência da distribuição potencial de habitats (*habitat-suitability modeling*) de mosquitos vetores de arbovírus no sudeste da Mata Atlântica. É discutida a possível importância desse método na vigilância das arboviroses no Brasil.

Abstract. Mosquitoes are vectors of arboviruses that can cause encephalitis and hemorrhagic fevers in humans. *Aedes serratus* (Theobald), *Aedes scapularis* (Rondani) and *Psorophora ferox* (Von Humboldt) are potential vectors of arboviruses and are abundant in Vale do Ribeira, located in the Atlantic Forest in the southeast of the State of São Paulo, Brazil. The objective of this study was to predict the spatial distribution of these mosquitoes and estimate the risk of human exposure to mosquito bites. Results of the analyses show that humans are highly exposed to bites in the municipalities of Cananéia, Iguape and Ilha Comprida. In these localities the incidence of Rocio encephalitis was 2% in the 1970s. Furthermore, *Ae. serratus*, a recently implicated vector of yellow fever virus in the State of Rio Grande do Sul, should be a target for the entomological surveillance in the southeastern Atlantic Forest. Considering the continental dimensions of Brazil and the inherent difficulties in sampling its vast area, the habitat suitability method used in the study can be an important tool for predicting the distribution of vectors of pathogens.

A comparison of 25 major infectious diseases in the world, which are the cause of highest mortality and/or morbidity among humans, showed that in the tropics, 8 of 10 are vector-borne diseases, whereas in temperate regions the ratio is

¹Laporta GZ, Ribeiro MC, Ramos DG, Sallum MAM. Spatial distribution of arboviral mosquito vectors (Diptera, Culicidae) in Vale do Ribeira in the South-eastern Brazilian Atlantic Forest. Cad Saude Publica. 2012; 28: 229-238.

2/15 (Wolfe et al., 2007). Furthermore, among the diseases that emerged globally from 1940 to 2004, approximately 23% were vector-borne and caused epidemics in tropical countries (Jones et al., 2008). Within this context, several previously unknown arboviruses were described from Amazon basin of Brazil in past decades (Rosa et al., 1998). Because of this, Brazil was considered a sanctuary for latent arboviruses (Bedin, 2007).

The Atlantic Forest possesses ecological and environmental conditions that support a large and diversified mosquito assemblage with species that can be vectors of both human and animal pathogens. Among those medically important mosquitoes, *Aedes scapularis* (Rondani), *Aedes serratus* (Theobald), and *Psorophora ferox* (Von Humboldt) were identified as potential vectors of arboviruses associated with severe human illnesses (Forattini et al., 1993; Forattini and Massad, 1998; Figueiredo, 2007). *Aedes scapularis* and *Ps. ferox* were incriminated as the vectors for the Rocio virus (ROCV), which caused 1,100 cases of encephalitis between 1975 and 1983 in the regions of Baixada Santista and Vale do Ribeira both in São Paulo State (Lopes et al., 1981; Mitchell and Forattini, 1984; Mitchell et al., 1986; Iversson, 1988). Additionally, *Ae. serratus* was found naturally infected with yellow fever virus (YFV) (Souza et al., 2010), and its role as a potential secondary vector of this virus in Rio Grande do Sul State during the epidemics and epizootics in 2008 (Cardoso et al., 2010) is possible.

The transmission of arboviruses is determined by the presence of a competent mosquito species and also by ecological interactions between reservoir / host, pathogens, and mosquito communities (Unnasch et al., 2006). Moreover, the presence and spatial distribution of a mosquito vector species is determined by both abiotic (e.g., temperature, water disposal, and topography) and biotic factors (e.g., abundance of vertebrate hosts and availability of larval habitats). These abiotic and biotic factors can be obtained from remotely sensed data and correlated to mosquito presence / abundance data (i.e., habitat-suitability model-

ling). This approach can be applied to infer the spatial distribution of a species in a variety of geographical scales (Ayala et al., 2009; Sinka et al., 2010). The starting point to perform habitat-suitability modelling is the framework proposed by Soberón (2007). This framework is based on the potential occupied area that is dependent on species niche determinants, as such: abiotic variables (e.g., climate, topography), biotic variables (e.g., vegetation cover) and metapopulation structure (e.g., source-sink dynamics). An example of the application of habitat-suitability modelling to surveillance and intervention activities is in the work by Diuk-Wasser et al. (2006) who generated maps to assess the spatial distribution of the West Nile virus mosquito vectors in New Haven County, Connecticut, USA.

In Atlantic Forest, ecological requirements for *Ae. scapularis*, *Ae. serratus*, and *Ps. ferox* are mainly temporary ground pools (larval habitats) and forest cover (shelter and host presences) (Forattini et al., 1993; Forattini and Massad, 1998) that can be measured by remote sensing technologies and thus utilized in the habitat-suitability modelling.

Considering the abundance of competent mosquito vectors (Forattini et al., 1993; Forattini and Massad, 1998), the circulation of several arboviruses (Calisher et al., 1982, 1983; Lopes et al., 1979) and the presence of natural ecosystems (dense ombrophilous forest, “restinga” forest, and mangrove; Ribeiro et al., 2009), the Vale do Ribeira may encompass pathogens that might accidentally emerge in humans. Although the role that some determinants of mosquito ecology can play on human epidemiology has already been studied in that region (Forattini et al., 1993; Forattini and Massad, 1998), the analyses were performed in a design of a spatially discrete scenario, which did not allow a quantitative extrapolation of the results. Nevertheless, habitat-suitability modelling permits extrapolation of the potential spatial distribution of mosquito vectors in continuous and quantitative geographical maps. Considering that the habitat-suitability modelling approach can be useful to the entomological surveillance system, the objectives

of the present study are: 1) to estimate the potential spatial distribution of *Ae. scapularis*, *Ae. serratus*, and *Ps. ferox* using habitat-suitability modelling; and 2) to identify municipalities where the humans are potentially more exposed to bites of *Ae. scapularis*, *Ae. serratus*, and *Ps. ferox* in Vale do Ribeira. Results of the present study may be useful to assess how potential spatial distribution maps can help the surveillance of vector-borne diseases in Brazil.

1.1 MATERIALS AND METHODS

1.1.1 Study area

The study encompassed 23 municipalities of Vale do Ribeira represented by 11,635 km² within the Serra do Mar, southeastern Brazil (Figura 1.1). The region comprises the largest continuous preserved forest of Atlantic Forest (Ribeiro et al., 2009), being characterized by a humid subtropical climate (Cfa in the Köppen classification), a hydrographic estuarine system, mosaics of human occupation, and a large assemblage of mosquito species (Forattini and Massad, 1998).

1.1.2 Mosquito collections

Adult sampling were carried out monthly from May 1996 to April 2000, in 9 collection sites (Figura 1.1), totalizing 108 independent collections. To determine the habitat-suitability for *Ae. scapularis*, *Ae. serratus*, and *Ps. ferox*, the total number of individuals of each species collected monthly in forest Shannon traps, 15:00-20:00 p.m., per collection site was used as a single variable. These values were further correlated to abiotic and biotic variables to assess the significant habitat-suitability predictors.

Figura 1.1: The map of the study area of Vale do Ribeira. The region is mainly covered with forest remnants in the Atlantic Forest biome. Mosquito collections were performed from nine sites. The reference grid is 23J with horizontal datum as WGS84 and vertical datum as Imbituba. (Source: SOS Mata Atlântica/INPE, 2008.)

1.1.3 Environmental variables

Topographic, climate, and landscape variables were quantified for each collection site using the GRASS 6.4 GIS software (<http://grass.osgeo.org/>). The variable slope derived from elevation map (<http://www2.jpl.nasa.gov/srtm/>; resampled to 30 m [Grohmann and Steiner, 2006]) was interpolated to 30 meters of spatial resolution and represented availability of larval habitats. Climate data had 900 meters of spatial resolution, and were acquired from the WorldClim database (<http://www.worldclim.org/>): mean maximum temperature in the warmest month, and annual precipitation. The landscape variable percentage of vegetation cover (30 meter-spatial resolution) was obtained by calculating the proportion of remaining vegetation within a radius of 200 m around each collection site. Similar approach was used in chironomid (Roque et al., 2010), bird (Boscolo and Metzger, 2009), and carnivore mammal (Lyra-Jorge et al., 2010) studies conducted in fragmented landscapes of São Paulo State. Vegetation map used in this step was derived from visual interpretation of Landsat TM imagery (Kronka et al., 2005).

1.1.4 Habitat suitability modelling

A preliminary analysis was performed to assess highly important abiotic and biotic determinants of the spatial distribution of a species. To determine if the contribution of each regression coefficient was significant ($p < 0.15$; equivalent to stepwise univariate regression α), the negative binomial regression analysis in the R 2.9.2 software (<http://www.r-project.org/>) was carried out. In this analysis, the total number of individuals of *Ae. scapularis*, *Ae. serratus*, and *Ps. ferox* were dependent variables, while the slope, temperature, precipitation, and vegetation were explanatory variables. This step allowed the assessment of the better correlated environmental variables for the habitat-suitability modelling.

Using the variables selected in the previous analysis, correlations between species presence data and environmental variables were assessed with the MAXENT algorithm (Phillips et al., 2006) of the OpenModeller 1.1 program (<http://openmodeller.sourceforge.net/>). As a result, the maximum entropy is statistically estimated. The predicted value for each pixel was employed to assess a species potential spatial distribution (30 meter-spatial resolution), which can vary from 0 (meaning the worst habitat) to 100 (the best habitat). The potential spatial distribution map, generated using the maximum entropy, is a result of a stochastic model and thus it needs to be validated. The receiver operating characteristic (ROC) method was applied to validate the models. A ROC plot was obtained for each species, by plotting all sensitivity values (e.g., mosquito presence fraction) against their equivalent (1 - specificity) values (e.g., mosquito absence fraction). To calculate both sensibility and specificity, we confronted predictions of mosquito presence and pseudo-absence against respectively mosquito presences and absences obtained from other field collections performed in the study region. The area under the ROC curve (AUC) provides a measure of overall accuracy (Fielding and Bell, 1997). The AUC values varies from 0 to 1. The former value means that the model fails in establishing habitat-suitability for a species, while the latter value indicates that the model is suitable. AUC values ≥ 0.90 were used herein as the cut-off for classifying a model as valid (Metz, 1986). In considering that a given species do not have a random spatial distribution because its habitat is not randomly distributed (Hanski, 1994); a second validation process was performed, based on the concept of metapopulation structure (Soberón, 2007). To do the validation, a Poisson distribution model was employed. A species potential spatial distribution map was validated when the habitat-suitability of a specific collection site (i.e., the population source) was higher than 80%. The 80% cut-off was defined because it represents those localities in which the species reached the optimum habitat.

A post-processing analysis was performed in which a zonal statistic, i.e., sum of values of pixels divided by the total number of pixels in a polygon (e.g., the boundary of each municipality), was applied in the spatial analyst Arcmap 10 program (<http://www.esri.com/>). The zonal statistic assigned mean pixel value of all the following rasters: slope, temperature, precipitation, and vegetation (environmental layers), *Ae. scapularis*, *Ae. serratus*, and *Ps. ferox* (potential spatial distribution maps) to all the 23 municipalities. Each of these 7 raster layers were transformed by the application of zonal statistic into 7 other variables with 23 values, i.e., mean pixel values for each municipality. The Spearman's rank correlation coefficient was employed to assess important predictors (slope, temperature, precipitation, or vegetation) to each potential spatial distribution map ($p < 0.20$). This *alpha* value ($p < 0.20$) was adopted here for the sake of simplicity, i.e., to avoid running multiple regression modelling after this long Materials and Methods section.

1.1.5 Epidemiological analysis

To estimate the man-biting rate, the potential spatial distribution map for each mosquito species (range: 0-100%) was transformed into a binary variable (0, absence and 1, presence), with a habitat-suitability cut-off of 80%. A mosquito presence in a 30 meter-spatial resolution was assumed to represent a proxy of the home range of a mosquito population. The predicted values of a mosquito presence were summed and divided by the number of inhabitants to represent the proportion of people at risk of being bitten. A value ≥ 1 means risk of human exposure to potentially infective mosquito bites.

1.2 RESULTS

A total of 14,835 females of *Ae. scapularis* (13,031), *Ae. serratus* (1,122), and *Ps. ferox* (692) were captured in Shannon traps in 9 localities of Vale do Ribeira. Total number of individuals of *Ae. scapularis*, *Ae. serratus*, and *Ps. ferox* did not fit a Poisson distribution, showing upper outliers that represented collection sites with suitable habitats for a given species. For each collection site, there were estimates of environmental variables that may determine variations on total number of individuals of *Ae. scapularis*, *Ae. serratus*, and *Ps. ferox* (Tabela 1.1). In the preliminary analysis, all abiotic (slope, temperature, and precipitation) and the biotic (vegetation) variables were selected to generate *Ps. ferox* and *Ae. serratus* potential distribution maps; precipitation layer was not employed in the modelling of *Ae. scapularis* because it was not significant (Tabela 1.2).

Potential spatial distribution maps of *Ae. scapularis* (range: 38-91%; AUC: 0.91; Figura 1.2A), *Ps. ferox* (range: 25-88%; AUC: 0.90; Figura 1.2B), and *Ae. serratus* (range: 24-86%; AUC: 0.90; Figura 1.2C) showed a habitat-suitability $\geq 80\%$ for the control sites, i.e., where the species have high probability to be present, Boqueirão Sul, Fazenda Experimental, and Pariquera-Mirim (*Ae. scapularis*) and Boqueirão Sul and Icapara (*Ps. ferox* and *Ae. serratus*) (Tabela 1.1, Figura 1.2).

In the study region, abiotic and biotic pixel values varied from 0 to 50° (slope), 1,400 to 2,792 mm (precipitation), 22.3 to 31.4°C (temperature), and 0 to 100% (vegetation). The highest mean values among municipalities were 13° of slope in Iporanga, 2,411 mm of precipitation in Cananéia, 30.7°C of temperature in Registro, and 94% of vegetation in Tapiraí. Potential *Ae. scapularis* spatial distribution was negatively correlated with slope ($\rho = -0.88$; $p < 0.001$; $n =$

Tabela 1.1: Collection sites, mosquito frequencies, and environmental variables in the Vale do Ribeira, São Paulo state, Brazil.

Sites	<i>Aedes</i> <i>scapularis</i>	<i>Aedes</i> <i>serratus</i>	<i>Psorophora</i> <i>ferox</i>	Terrain slope (°)	Average annual precip- itation (mm)	Average maximum temperature in the warmest month (°C)	Vegetation cover (%)
BoqN	1,073	89	72	0	2,116	29.9	15
BoqS	2,244 ^a	368 ^a	209 ^a	1	2,279	30.5	64
CanSJ	831	99	58	9	2,469	30.6	27
CanT	646	48	22	13	2,272	30.2	77
FazE	4,157 ^a	46	6	2	1,915	30.9	3
SGa	528	61	30	4	2,043	30.5	81
IcaI	887	287 ^a	227 ^a	0	2,096	29.5	93
Par	1,899 ^a	90	55	1	2,060	30.6	0
VM	756	34	13	0	2,012	30.8	0

^a: higher mosquito frequency as expected in a Poisson distribution. These collections sites were herein considered as the source of a mosquito-specific species, i.e., a location with ecological characteristics that positively influence the population growth. These localities were the control sites in where the species are present in the context of habitat-suitability modelling.

Mosquito collection sites: BoqN: Ilha Comprida (North); BoqS: Ilha Comprida (South); CanSJ: Cananéia; CanT: Sítio Itapuã; FazE: Fazenda Experimental; SGa: Sítio Galiléia; IcaI: Icapara; Par: Pariquera-Mirim; VM: Vila Maria.

Tabela 1.2: Preliminary analysis: significance of coefficient ($p < 0.15$) in negative binomial regression.

Species	Terrain slope (°)	Average annual precipitation (mm)	Average maximum temperature in the warmest month (°C)	Vegetation cover (%)
<i>Aedes scapularis</i>	$\beta_1 = -0.07, p < 0.11$	$\beta_1 = -0.001, p < 0.19^a$	$\beta_1 = 0.08, p < 0.09$	$\beta_1 = -0.01, p < 0.07$
<i>Psorophora ferox</i>	$\beta_1 = -0.10, p < 0.13$	$\beta_1 = 0.003, p < 0.13$	$\beta_1 = -0.14, p < 0.04$	$\beta_1 = 0.02, p < 0.03$
<i>Aedes serratus</i>	$\beta_1 = -0.08, p < 0.13$	$\beta_1 = 0.002, p < 0.12$	$\beta_1 = -0.09, p < 0.12$	$\beta_1 = 0.013, p < 0.02$

^a: this variable was not selected to potential habitat suitability modelling of the given species under the null hypotheses ($\beta_1 = 0$; $p < 0.15$).

Figura 1.2: *Aedes scapularis* (A; AUC: 0.91; range: 38-91%), *Psorophora ferox* (B; AUC: 0.90; range: 25-88%), and *Aedes serratus* (C; AUC: 0.90; range: 24-86%) habitat-suitability maps. Control sites have habitat suitability $\geq 80\%$. The zoom in each map is to show that all control sites (see Tabela 1.1) have habitat suitability $\geq 80\%$.

Tabela 1.3: Risk of human exposure to mosquito vectors in the Vale do Ribeira, São Paulo state, Brazil.

Municipalities	<i>Aedes scapularis</i>	<i>Aedes serratus</i>	<i>Psorophora ferox</i>
Cananéia	28.47(349,188 ^a /12,267 ^b)	69.03(846,781/12,267)	68.91(845,322/12,267)
Iguape	46.23(1,267,072/27,410)	31.93(875,152/27,410)	42.11(1,154,344/27,410)
Ilha Comprida	18.23(121,266/6,653)	16.03(106,619/6,653)	16.74(111,367/6,653)
Iporanga	0.72(3,296/4,563)	0.70(3,202/4,563)	0.48(2,197/4,563)

^a: number of pixels in which species is present (habitat suitability $\geq 80\%$).

^b: human population in the 2000 IBGE census (<http://www.ibge.gov.br/>).

23) and positively correlated with precipitation ($\rho = 0.61$; $p < 0.002$; $n = 23$) and temperature ($\rho = 0.52$; $p < 0.02$; $n = 23$). Potential *Ps. ferox* and *Ae. serratus* spatial distributions were negatively correlated with slope ($\rho = -0.31$ and -0.24 ; $p < 0.15$ and 0.27 ; $n = 23$; respectively) and were positively correlated with precipitation ($\rho = 0.77$ and 0.78 ; both $p < 0.001$; $n = 23$; respectively) and temperature ($\rho = 0.63$ and 0.58 ; $p < 0.002$ and 0.004 ; $n = 23$; respectively). Interestingly, correlations between vegetation and potential *Ae. serratus* and *Ps. ferox* spatial distributions were stronger ($\rho = 0.36$ and 0.29 ; $p < 0.10$ and 0.19 ; $n = 23$; respectively) than with potential *Ae. scapularis* spatial distribution ($\rho = 0.12$; $p < 0.58$; $n = 23$). According to these results, areas with greater slope, which are usually on the mountains (e.g., Iporanga), will represent less-suitable habitats, whereas areas on the coast (e.g., Cananéia) and on the plain (e.g., Registro) will show high values of precipitation and temperature, which configure suitable habitats for these mosquitoes. Therefore, Cananéia, Iguape, and Ilha Comprida municipalities had the highest risk of human exposure to mosquito bites, whereas inhabitants of Iporanga municipality are not exposed to these mosquitoes (Tabela 1.3).

1.3 DISCUSSION

Habitat suitability modeling was applied for extrapolating the potential spatial distribution of each species in all municipalities of Vale do Ribeira. The word “potential” is intentionally placed before “spatial distribution” in order to better inform what these maps represent. Both spatial and temporal dimensions were considered for the study design when field collections started in May 1996. One objective of this study design, which was part of a Thematic Project (FAPESP no. 95/03273-8; Dr. Forattini coordinated this research project), was to assess associations between local environmental characteristics and emergence of mosquito vectors in natural, rural and sub-urban landscapes in Vale do Ribeira. Here, we reused this dataset to represent a common situation in the mosquito surveillance in which data is usually scarce.

Potential spatial distribution maps for *Ae. scapularis* (Figura 1.2A), *Ps. ferox* (Figura 1.2B), and *Ae. serratus* (Figura 1.2C) indicate that these species are largely distributed in Vale do Ribeira, as shown by Forattini et al. (1993) and Forattini and Massad (1998). Abiotic and biotic determinants were applied with a habitat-suitability modelling to obtain geographical maps that might represent the potential niche, i.e., locations favorable for occupation by a species (Soberón, 2007). However, an external validation may be necessary, i.e., a sampling effort in highly favorable areas predicted by the potential spatial distribution models. Despite this, the models can be improved as this study area has been sampled over the time.

The results found here are in accordance with the ecological characteristics of mosquito species considered and the distribution of the cases of Rocio virus (ROCV) encephalitis in Vale do Ribeira. According to correlation tests and Figura 1.2B, *Ps. ferox* is present along coastal humid lowlands (Forattini et al., 1993) and might have played a role in enzootic cycle maintenance of ROCV during

the 1976 epidemic (Lopes et al., 1981) in the municipalities of Cananéia, Iguape, and Ilha Comprida (Tabela 1.3). The non-significant correlation between potential *Ae. scapularis* spatial distribution (Figura 1.2A) and vegetation cover ($p < 0.58$) showed that this species has a tendency to be present in environments that were modified by human activities (Forattini and Massad, 1998). This mosquito has a high vector competence to transmit the ROCV among humans (Mitchell and Forattini, 1984; Mitchell et al., 1986). Interestingly, humans are highly exposed to this mosquito in Cananéia, Iguape, and Ilha Comprida (Tabela 1.3), which is in accordance with the high incidence rate (2%) of Rocio virus encephalitis in 1976 (Iversson, 1988). Both *Ps. ferox* and *Ae. scapularis* played roles as vectors of ROCV in 1970 and 1980 decades in these municipalities and thus these mosquitoes need constant attention.

The risk of yellow fever virus (YFV) transmission still remains low in Atlantic Forest (Souza et al., 2010). However, individuals of *Ae. serratus* were naturally found infected by the YFV, other arboviruses were isolated from this species in Amazon basin (Segura and Castro, 2007), and humans are highly exposed to this mosquito in Vale do Ribeira (Tabela 1.3). Unfortunately, the role of the species in the epidemiology of yellow fever is unknown. Although potential *Ae. serratus* spatial distribution represents a past scenario (1996-2000), it is possible to assume that it can be extrapolated to the present because only minor vegetation cover changes occurred from the 1980s (58% in Iversson, 1988) to the 2000s (53% in Ribeiro et al., 2009). This assumption is based on the correlation between this species and vegetation cover ($\rho = 0.36$; $p < 0.10$). Moreover, Pardini et al. (2010) showed that the abundance of specialist species may not respond to vegetation loss in a fragmented landscape with $\geq 50\%$ remaining forest.

Potential spatial distribution maps of vectors are important for the surveillance of arboviruses in Brazil, where dormant or nonpathogenic viruses can emerge because of densely populated cities infested by mosquitoes (Bedin, 2007;

Figueiredo, 2007). According to Wolfe et al. (2007) and Jones et al. (2008), Vale do Ribeira region seems to favor the emergence of vector-borne diseases. The results from the habitat-suitability modelling showed that careful attention should be given to the mosquito assemblages in Vale do Ribeira, and mainly in the municipalities of Cananéia, Iguape, and Ilha Comprida. Although a similar recommendation was made by Forattini and Massad (1998), the major contribution of the present study was to apply the habitat-suitability modelling as a tool for estimating the species potential spatial distributions, which can contribute for mosquito surveillance, as demonstrated in North America by Diuk-Wasser et al. (2006).

Estimates of the number of people at risk considered total population but did not take account of immune responses to arbovirus infection. It is possible that a fraction of people might have become refractory to a new infection because of past exposition. Therefore, this fraction of immunes should also be considered for increasing accuracy of estimates of people at risk.

Considering that the habitat-suitability modelling can be applied in broader areas than the current extent of the mosquito collection sites, this approach is recommendable in tropical regions (mainly Latin America), where sampling data are not often available. We believe that the potential spatial distribution maps presented here can contribute to define strategic areas to monitor mosquito populations and to evaluate the risk of arboviruses emergence in Brazil, optimizing the use of resources and also minimizing efforts spent in the surveillance. Combined with assessments of the presence of viruses in mosquito samples, habitat-suitability modelling can be applied in surveillance programs, providing elements for risk assessment, decision-making and adoption of preventive measures.

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Capítulo 2

Habitat suitability of *Anopheles* vectors in the Vale do Ribeira

Esse capítulo foi publicado nas Memórias do Instituto Oswaldo Cruz¹. Foi aplicado modelo de distribuição potencial de habitats para estimar a distribuição espacial de mosquitos vetores de plasmódios no sudeste da Mata Atlântica. As distribuições de *Anopheles cruzii* and *Anopheles marajoara* estão positivamente correlacionadas com casos autóctones de malária, o que sugere dois focos de transmissão: um em que o plasmódio circula entre primatas e *An. cruzii* e outro no qual o parasito pode circular entre *An. marajoara* e humanos. É discutida a relação dessa evidência (i.e., focos maláricos silvestre e rural) no uso do solo e conservação da Mata Atlântica.

Abstract. Every year, autochthonous cases of *Plasmodium vivax* malaria occur in low-endemicity areas of Vale do Ribeira in the south-eastern part of the Atlantic Forest, state of São Paulo, where *Anopheles cruzii* and *Anopheles bellator* are considered the primary vectors. However, other species in the subgenus *Nyssorhynchus* of *Anopheles* (e.g., *Anopheles marajoara*) are abundant and may participate in the dynamics of malarial transmission in that region. The objectives of the present study were to assess the spatial distribution of *An. cruzii*, *An. bellator* and *An. marajoara* and to associate the presence of these species with malaria cases in the municipalities of the Vale do Ribeira. Potential habitat suitability modelling was applied to determine both the spatial distribution of *An. cruzii*, *An. bellator* and *An. marajoara* and to establish the density of each species. Poisson regression was utilized to associate malaria cases with estimated vector densities. As a result, *An. cruzii* was correlated with the forested slopes of the Serra do Mar, *An. bellator* with the coastal plain and *An. marajoara* with the deforested areas. Moreover, both *An. marajoara* and *An. cruzii* were positively associated with malaria cases. Considering that *An. marajoara* was demonstrated to be a primary vector of human *Plasmodium* in the rural areas of the state

¹Laporta GZ, Ramos DG, Ribeiro MC, Sallum MAM. Habitat suitability of *Anopheles* vector species and association with human malaria in the Atlantic Forest in south-eastern Brazil. Mem Inst Oswaldo Cruz. 2011; 106(Suppl. I): 239-245.

of Amapá, more attention should be given to the species in the deforested areas of the Atlantic Forest, where it might be a secondary vector.

Land use change, deforestation and agricultural expansion are human modifications of the natural environment, which have had direct effects on the exacerbation of malaria in the Amazon basin (Castro et al., 2006; Vittor et al., 2006; Olson et al., 2010). Thousands of malaria cases occur annually in areas of active transmission in Brazil; however, a few autochthonous cases are reported in localities outside the Amazon region. In the forested areas of the Atlantic coast, malarial transmission is low and irregular (Oliveira-Ferreira et al., 2010). Couto et al. (2010) reported that 444 cases of *Plasmodium vivax*-malaria occurred in the Vale do Ribeira on the south-eastern coast of the state of São Paulo (SP) between 1980-2007. Moreover, nonhuman primates (e.g., *Alouatta guariba clamitans* Cabrera) were found to be infected by *P. vivax*, *Plasmodium malariae* and *Plasmodium falciparum* (Curado et al., 2006; Duarte et al., 2008) in that region. In the forested areas of Vale do Ribeira, *Anopheles (Kerteszia) cruzii* Dyar and Knab and *Anopheles (Kerteszia) bellator* Dyar and Knab are vectors of malaria parasites (Forattini et al., 1999; Marrelli et al., 2007). In considering that the presence of *An. cruzii* and *An. bellator* may be associated with *vivax* malaria transmission in the Atlantic Forest, it would be important to determine the potential spatial distribution of both vector species, mainly in under-sampled areas of the Vale do Ribeira.

Species of the subgenus *Nyssorhynchus* may also be involved in the dynamics of malarial transmission in areas outside the Amazon Region. Among the *Nyssorhynchus* species, it is worth mentioning *Anopheles marajoara* Galvão and Damasceno of the *Anopheles albitarsis* complex because this species is a primary vector of the human *Plasmodium* sp. in Macapá, state of Amapá, north-eastern Amazon. In that region, *An. marajoara* has replaced *Anopheles darlingi* Root as the primary vector and that event was associated with changes in the land

use (Conn et al., 2002). Furthermore, species of the *An. albitarsis* complex may be involved in the dynamics of malarial transmission in certain areas of the Atlantic Forest in south-eastern Brazil. For example, *An. marajoara* was the primary vector in the municipality of Iguape, Vale do Ribeira, in the 1940s (Schiavi, 1945). The author pointed out that Iguape did not provide any suitable marshy habitat for the immature members of this species and also suggested that the adults emerged from the larval habitats in Ilha Comprida, which is separated from the continent by an estuarine complex (i.e., Mar Pequeno). Considering the epidemiological importance of the species, Correa et al. (1950) conducted a mark-release-recapture experiment to verify the dispersion of *Anopheles albitarsis domesticus* (= *An. marajoara*) between Ilha Comprida and Iguape and vice-versa. Marked specimens released in Iguape were found in Ilha Comprida, whereas specimens from Ilha Comprida were captured in Iguape. Wilkerson et al. (1995), employing randomly amplified polymorphic DNA polymerase chain reaction, confirmed the presence of *An. marajoara* in Ilha Comprida, whereas *An. albitarsis* s.s. and *An. albitarsis* B (i.e., *Anopheles oryzalimnetes* Wilkerson and Motoki) were found in the municipality of Registro, in the Vale do Ribeira (Motoki et al., 2009). *An. marajoara* was the only species of the *An. albitarsis* complex present in Ilha Comprida, which corroborates with Schiavi (1945) and Correa et al. (1950). However, the distribution of *An. marajoara* in other areas of the Vale do Ribeira is unknown mainly because of under-sampling as well as problematic species identification based mainly on morphological characteristics. Considering the importance of *An. marajoara* as a primary vector of human *Plasmodium* in Macapá (Conn et al., 2002), it is reasonable to assume that this species may be involved in the dynamics of human malarial transmission in areas outside the Amazon, such as in the Vale do Ribeira.

The distribution of *An. marajoara* may have been underestimated by Wilkerson et al. (1995) because some areas of the Vale do Ribeira possess adequate

habitat and ecological conditions for the proliferation of the *An. albitarsis* species complex. For example, Forattini et al. (1995) demonstrated that irrigated rice fields provide a suitable habitat for the immature *An. albitarsis* l.s. Consequently, to establish the potential distribution of *An. marajoara* in the Vale do Ribeira, it may be helpful to understand the spatial dynamics of human malaria in areas of low-endemicity in the Atlantic Forest.

Considering that the spatial distributions of the five primary vectors of human *Plasmodium* are not completely known in Africa, Ayala et al. (2009) applied predictive distribution modelling based on the presence of a species for constructing habitat suitability maps for *Anopheles gambiae* Giles, *Anopheles funestus* Giles, *Anopheles arabiensis* Patton, *Anopheles nili* (Theobald), and *Anopheles moucheti* Evans in Cameroon. *An. gambiae* and *An. funestus* were ecologically more tolerant, whereas *An. arabiensis*, *An. moucheti* and *An. nili* were more specialized. A similar approach was employed in the Malaria Atlas Project to establish the potential distributions of the dominant mosquito vector species of human *Plasmodium* (Hay et al., 2010; Sinka et al., 2010).

Considering that the majority of the Atlantic Forest is under-sampled for mosquito assemblages, ecologic niche modelling is a powerful tool that can both predict the potential spatial distribution of the vectors and assess the potential risk of exposure to human *Plasmodium* sp. circulating in that region. The objectives of the present study are: (i) to apply habitat suitability models to predict the potential spatial distribution of *An. bellator*, *An. cruzii* and *An. marajoara*; (ii) to associate autochthonous cases of human malaria with the potential spatial distribution of these mosquitoes in the Vale do Ribeira in south-eastern Atlantic Forest, Brazil.

Figura 2.1: Twenty three municipalities of the study area Vale do Ribeira, south-eastern of the state of São Paulo, Brazil. The region is mainly covered with forest remnants in the Atlantic Forest biome. Mosquito collections were performed from nine sites. MG: state of Minas Gerais; MS: state of Mato Grosso do Sul; PR: state of Paraná; RJ: state of Rio de Janeiro; SC: state of Santa Catarina; SP: state of São Paulo. The reference grid is 23J with horizontal datum as WGS84 and vertical datum as Imbituba. (Source: SOS Mata Atlântica/INPE, 2008.)

2.1 MATERIALS AND METHODS

2.1.1 Study area

The study encompassed 23 municipalities of the Vale do Ribeira, which occupies 11,635 km² within the Serra do Mar in south-eastern Brazil (Figura 2.1). The region comprises the largest continuous preserved forest (approximately 1,100 million ha) in the Atlantic Forest biome (Ribeiro et al., 2009), with a hydrographic estuarine system that is intermixed with mosaics of human occupation, as well as a rich assemblage of mosquito species (Forattini and Massad, 1998). The climate is characterized as humid subtropical (Cfa in the Köppen classification).

2.1.2 Mosquito collections

Adult mosquito sampling was carried out monthly from May 1996-April 2000 in nine collection sites (Figura 2.1) totaling 108 sampling units. To determine the habitat suitability for *An. cruzii*, *An. bellator* and *An. marajoara*, the total number of individuals of each species collected in forest Shannon traps, 15:00-20:00 p.m., each month at each collection site was used as a single measurement. These values were further correlated to abiotic and biotic variables to assess the predictors of potential habitat suitability.

2.1.3 Environmental variables

Terrain slope, bioclimatic variables and vegetation cover were the habitat variables that were quantified for each collection site by applying distinct routines in the GRASS 6.4 GIS software. These variables were assumed to represent ecologic niches because each species is potentially adapted for specific ranges of

the habitat variables. The topographically variable terrain slope, derived from the Shuttle Radar Topography Mission (SRTM) 1.4 elevation map (resampled to 30 m [Grohmann e Steiner, 2006]), was interpolated to a 30-meter spatial resolution, which was assumed to be the home range of a mosquito population. Climate data from 1950-2000 with a 900-meter spatial resolution were acquired from the WorldClim database.

For the purpose of the analyses, the average maximum temperature in the warmest month was defined as Bio5, and the average annual precipitation as Bio12. Although the area encompassed in a 900-meter survey is bigger than that assumed for the mosquito population, the climate variables represented an accurate approximation for the study area. The accuracy was assessed by comparing Bio5 and Bio12 estimates to the values obtained from the local meteorological stations in the municipalities of Registro and Pariquera-Açu. The landscapes were characterized by the percentage of vegetation cover estimated within a 200-meter radius around each sampling site. A similar approach was used for chironomids (Roque et al., 2010), birds (Boscolo and Metzger, 2009) and carnivore mammals (Lyra-Jorge et al., 2010) in the fragmented landscapes of SP. A natural vegetation map was derived from the visual interpretation of a 30-meter spatial resolution Landsat-TM imagery (Kronka et al., 2005).

2.1.4 Habitat suitability modelling

To select statistically significant abiotic and biotic variables, which can explain the potential habitat suitability of each species, a preliminary analysis was performed. In this work, the number of individuals of *An. bellator*, *An. cruzii* and *An. marajoara* were defined as the dependent variables, whereas terrain slope, Bio5, Bio12 and vegetation cover were the independent variables. Subsequently,

negative binomial regressions were run with the software R-2.9.2, with the MASS package, to determine if the contribution of each variable was significant ($p < 0.15$; equivalent to stepwise univariate regression α).

Using the variables selected in the aforementioned steps, the potential habitat suitability for *An. bellator*, *An. cruzii* and *An. marajoara* was estimated by employing the MAXENT algorithm (Phillips et al., 2006) implemented in the openModeller 1.1.0 software. Firstly, the MAXENT was used to estimate the coefficient value of the presence of a mosquito species as predicted by the prevailing abiotic and biotic variables. Then the coefficient values were used in 500-iteration runs to estimate the maximum likelihood (ML) of an expected binomial distribution (0, pseudo-absence; 1, presence). The ML probability of the presence of a species was estimated for each collection site, which represented the potential habitat suitability of this species. These values varied from 0% for an unsuitable habitat to 100% for suitable habitats and were used to construct maps with a 30-meter spatial resolution that covered the entire Vale do Ribeira region. The receiver operating characteristic (ROC) method was used to validate the potential habitat suitability maps, which were generated under stochastic modelling. The area under the ROC curve (AUC) provides a measure of accuracy (Fielding and Bell, 1997). The AUC values varied from 0-1, where 0 meant that the model failed to establish the potential habitat suitability of a species, while a score of 1 indicated that the selected model was accurate. An AUC value of 0.9 was used as the cut-off for determining model adequacy (Metz, 1986). In considering that a given species did not have a random spatial distribution because its habitat was not randomly distributed (Hanski, 1994), a second validation process was performed based on the concept of a metapopulation structure (Soberón, 2007). To do this validation, a Poisson distribution model was used. A species potential distribution map was considered as validated when the potential habitat suitability of a specific collection site was higher than 80%. An 80% cut-off was used as

a standard because it represented those localities in which the species reached the optimum habitat and thus could be involved in the transmission of *Plasmodium*.

The terrain slope, Bio5, Bio12, vegetation cover and potential habitat suitability of *An. bellator*, *An. cruzii* and *An. marajoara* data were extrapolated for the entire region of the Vale do Ribeira. The extrapolation was conducted by correlating potential habitat suitability with abiotic and biotic variables, which were aggregated for each municipality. The mean pixel values of each variable were estimated by using the spatial analyst extension of Arcmap 10 software. The Spearman's rank test was used to estimate the correlation coefficient between the pixel mean of potential habitat suitability (dependent variable) and each abiotic and biotic variable ($p < 0.10$). This *alpha* value ($p < 0.10$) was adopted here for the sake of simplicity, i.e., to avoid running multiple regression modelling.

2.1.5 Epidemiological analysis

Finally, an association between the prevalence of mosquito bites and malaria cases was assessed. Prevalence to mosquito bites was assumed to be positively linked to the potential habitat suitability values. Poisson regressions were applied using the numbers of autochthonous cases of malaria that were registered for each municipality from 1996-2000 as dependent variables. The number of malaria cases was obtained from Couto et al. (2010). Potential habitat suitability values aggregated per municipality were transformed into a binary variable (0 = absence; 1 = presence) for each species and later used as independent variables. A species was considered present in a specific locality when the value of the habitat suitability was $\geq 80\%$, whereas a $< 80\%$ cut-off value meant absence. Cases of malaria were tested to ascertain whether they had a Poisson distribution using the Moran Global Autocorrelation test. Considering that malaria cases have a Poisson distribution, the regression coefficients were estimated using a generalized

linear model to estimate the prevalence ratio (PR) of the disease associated with multiple independent variables. The variables were altered into dummy variables as follow: $X_1 = An. bellator$ (model 1); $X_2 = An. cruzii$ (model 2); $X_3 = An. marajoara$ (model 3); $X_4 =$ both *An. bellator* and *An. cruzii* (model 4); $X_5 =$ both *An. bellator* and *An. marajoara* (model 5); $X_6 =$ both *An. cruzii* and *An. marajoara* (model 6); $X_7 = An. bellator, An. cruzii$ and *An. marajoara* (model 7). The PR and the confidence interval (95%) were estimated in the software R-2.9.2 with the *epicalc* package to identify mosquito species that were positively associated with malaria ($PR > 1$; $p < 0.05$).

2.2 RESULTS

A total of 8,288 females of *An. bellator* (2,700), *An. cruzii* (5,428) and *An. marajoara* (160) were captured. Collection sites, mosquito species and both abiotic and biotic variables are shown in Tabela 2.1. The mean and standard deviations are in the Tabela 2.2. The total number of mosquitoes showed a negative binomial distribution in which abiotic and biotic variables estimated the coefficients of the regressions. The significant coefficient indicated that the terrain slope and vegetation cover (*An. cruzii*), Bio12 and vegetation cover (*An. bellator*) and Bio5 and vegetation cover (*An. marajoara*) were the most explicative variables; thus they were selected for the potential habitat suitability modelling (Tabela 2.3).

Potential habitat suitability maps for *An. bellator* (range: 4-88%, AUC: 0.95), *An. cruzii* (range: 51-97%, AUC: 0.91) and *An. marajoara* (range: 3-95%, AUC: 0.95) are shown in the Figura 2.2A, 2.2B, and 2.2C, respectively. Collection sites in which the numbers of mosquitoes were higher than that expected within a

Figura 2.2: Potential habitat suitability maps *Anopheles bellator* (A), *Anopheles cruzii* (B), *Anopheles marajoara* (C) potential habitat suitability maps, and cases of malaria (D) in the south-eastern Atlantic Forest of Brazil. Potential habitat suitability of *An. cruzii* is beyond the slopes of Serra do Mar.

Tabela 2.1: Collection sites, mosquito species and abiotic and biotic variables in the south-eastern Atlantic Forest of Brazil

Sites	<i>Anopheles bellator</i>	<i>Anopheles cruzii</i>	<i>Anopheles marajoara</i>	Terrain slope (°)	Average annual precipitation (mm)	Average maximum temperature in the warmest month (°C)	Vegetation cover (%)
BoqN	18	194	126 ^a	0	2,116	29.9	15
BoqS	1,796 ^a	509	21	1	2,279	30.5	64
CanSJ	523 ^a	454	4	9	2,469	30.6	27
CanT	316	2,751 ^a	0	13	2,272	30.2	77
FazE	0	352	9	2	1,915	30.9	3
SGa	11	611	0	4	2,043	30.5	81
IcaI	36	543	0	0	2,096	29.5	93
Par	0	14	0	1	2,060	30.6	0
VM	0	0	0	0	2,012	30.8	0

^a: higher mosquito frequency as expected in a Poisson distribution. These collection sites were herein considered as the source of a mosquito-specific species, i.e., a location with ecological characteristics that positively influence the population growth. These localities were the control sites utilized for the validation of potential habitat suitability modelling.

Mosquito collection sites: BoqN: Ilha Comprida (North); BoqS: Ilha Comprida (South); CanSJ: Cananéia; CanT: Sítio Itapuã; FazE: Fazenda Experimental; SGa: Sítio Galiléia; IcaI: Icapara; Par: Pariquera-Mirim; VM: Vila Maria.

Poisson distribution were then assumed to be population sources. Boqueirão Sul and Morro do São João (*An. bellator*), Sítio Itapuã (*An. cruzii*) and Boqueirão Norte (*An. marajoara*) (Tabela 2.1) showed $\geq 80\%$ potential habitat suitability for *An. bellator* (Figura 2.3A), *An. cruzii* (Figura 2.3B) and *An. marajoara* (Figura 2.3C). Abiotic and biotic values varied from 0-50° (terrain slope), 1,400-2,792 mm (Bio12), 22.3-31.4°C (Bio5) and 0-100% (vegetation cover). The higher values were 13° of terrain slope in Iporanga, 2,411 mm of Bio12 in Cananéia, 30.7°C of Bio5 in Registro and 94% of vegetation cover in Tapiraí. *An. cruzii* potential habitat suitability was positively correlated to terrain slope ($\rho = 0.93$, $p < 0.001$, $n = 23$), high vegetation cover (e.g., 86% in Iporanga), low Bio12 (e.g., 1,602 mm in Iporanga) and Bio5 (e.g., 25.6°C in Juquitiba). *An. bellator* was positively correlated to Bio5 ($\rho = 0.62$, $p < 0.002$, $n = 23$) and Bio12 ($\rho =$

Tabela 2.2: The mean and standard deviations of the number of mosquitoes monthly collected per species and collection site in the south-eastern Atlantic forest of Brazil

Sites	<i>Anopheles bellator</i>	<i>Anopheles cruzii</i>	<i>Anopheles marajoara</i>
BoqN	1.50±1.62	16.17±5.25	10.50±4.17
BoqS	149.67±17.08	42.42±11.24	1.75±1.55
CanSJ	43.58±8.03	37.83±10.18	0.33±0.49
CanT	26.34±7.20	229.25±19.24	0.00±0.00
FazE	0.00±0.00	29.33±9.86	0.75±0.75
SGa	0.92±1.17	50.92±9.62	0.00±0.00
IcaI	3.00±2.26	45.25±10.53	0.00±0.00
Par	0.00±0.00	1.17±1.47	0.00±0.00
VM	0.00±0.00	0.00±0.00	0.00±0.00

Mosquito collection sites: BoqN: Ilha Comprida (North); BoqS: Ilha Comprida (South); CanSJ: Cananéia; CanT: Sítio Itapuã; FazE: Fazenda Experimental; SGa: Sítio Galiléia; IcaI: Icapara; Par: Pariquera-Mirim; VM: Vila Maria.

Tabela 2.3: Preliminary analysis: equations of negative binomial regression models and significance of coefficient β_1 of the independent variable X_1 ($p < 0.15$)

Species	Terrain slope (°)	Average annual precipitation (mm)	Average maximum temperature in the warmest month (°C)	Vegetation cover (%)
<i>An. bellator</i>	$5.59 + 0.03 * X_1$	$-7.09 + 0.006 * X_1$	$-45.29 + 0.17 * X_1$	$3.20 + 0.05 * X_1$
Coefficient β_1	$z=0.17; p<0.87^a$	$z=49.88; p<0.01^b$	$z=0.86; p<0.40^a$	$z=2.37; p<0.02^b$
<i>An. cruzii</i>	$5.39 + 0.18 * X_1$	$-1.80 + 0.004 * X_1$	$44.83 - 0.13 * X_1$	$4.88 + 0.03 * X_1$
Coefficient β_1	$z=69.47; p<0.01^b$	$z=1.27; p<0.21^a$	$z=-1.01; p<0.28^a$	$z=0.03; p<0.02^b$
<i>An. marajoara</i>	$3.36 - 0.34 * X_1$	$4.16 - 0.001 * X_1$	$49.77 - 0.16 * X_1$	$3.40 - 0.04 * X_1$
Coefficient β_1	$z=-1.35; p<0.18^a$	$z=-0.10; p<0.92^a$	$z=-9.5; p<0.01^b$	$z=-1.56; p<0.12^b$

^a: this variable was not selected to potential habitat suitability modelling of the given species ($p > 0.15$). X_1 : independent variable *An. bellator*, *An. cruzii* and *An. marajoara*.

^b: These results represent significant outcomes under the null hypotheses ($\beta_1 = 0; p < 0.15$).

Tabela 2.4: Poisson regression of malaria cases in function of binary potential habitat suitability $\geq 80\%$ (presence)/ $< 80\%$ (absence)

Models (Malaria cases $\sim$ Poisson)	PR (95%CI)	p (Wald's test)
1: Malaria cases $\sim$ Poisson ($\lambda = e^{-0.31+0.43*X_1}$)	1.53 (0.64-3.7)	p < 0.35
2: Malaria cases $\sim$ Poisson ($\lambda = e^{-0.00-0.16*X_2}$)	0.85 (0.25-2.9)	p < 0.80
3: Malaria cases $\sim$ Poisson ($\lambda = e^{-2.2+2.50*X_3}$) ^a	12.21 (1.64-91.24)	p < 0.02
4: Malaria cases $\sim$ Poisson ($\lambda = e^{-0.25+0.43*X_4}$)	1.54 (0.59-4.01)	p < 0.39
5: Malaria cases $\sim$ Poisson ($\lambda = e^{-0.31+0.43*X_5}$)	1.53 (0.64-3.7)	p < 0.35
6: Malaria cases $\sim$ Poisson ($\lambda = e^{-1.01+1.47*X_6}$) ^a	4.36 (1.46-13.05)	p < 0.01
7: Malaria cases $\sim$ Poisson ($\lambda = e^{-0.25+0.43*X_7}$)	1.54 (0.59-4.01)	p < 0.39

^a: significant model, prevalence ratio (PR) > 1 and (p < 0.05). Independent variables: X_1 : *Anopheles bellator*; X_2 : *Anopheles cruzii*; X_3 : *Anopheles marajoara*; X_4 : both *An. bellator* and *An. cruzii*; X_5 : both *An. bellator* and *An. marajoara*; X_6 : both *An. cruzii* and *An. marajoara*; X_7 : *An. bellator*, *An. cruzii* and *An. marajoara*; CI: confidence interval.

The results printed in bold represent significant outcomes under the null hypotheses (PR = 1; p < 0.05).

0.76, p < 0.001, n = 23) and negatively correlated to terrain slope (rho = -0.43, p < 0.05, n = 23). *An. marajoara* was positively correlated to Bio5 (rho = 0.93, p < 0.001, n = 23) and negatively correlated to vegetation cover (rho = -0.35; p < 0.10, n = 23).

Figura 2.3: Binary potential habitat suitability maps (control sites have potential habitat suitability $\geq 80\%$). A: *Anopheles bellator* [area under the receiver operating characteristic curve (AUC) 0.95, range 4-88%]; B: *Anopheles cruzii* (AUC 0.91, range 51-97%); C: *Anopheles marajoara* (AUC 0.95, range 3-95%). The zoom in each map is to show that all control sites (see Tabela 2.1) have habitat suitability $\geq 80\%$.

Malaria cases followed a Poisson distribution in which spatial autocorrelation was not significant (Moran Z score = 1.71, $p < 0.09$). The disease was distributed by municipalities with the following frequencies: one case each in Cananéia, Pariqueira-Açu, Registro, Juquiá and Tapiraí, two cases each in Iguape and Pedro de Toledo, three in Miracatu and eight in Sete Barras (Figura 2.2D). Associations between malaria and dummy variables showed that models 1, 4, 5 and 7, which include *An. bellator* as a vector, were not significant. However, models 3 (= malaria cases $\sim$ *An. marajoara*) and 6 (= malaria cases $\sim$ both *An. cruzii* and *An. marajoara*) were significant, showing associations between exposure to those mosquitoes and disease. The values obtained were 12.21 (1.64-91.24, $p < 0.02$) and 4.36 (1.46-13.05, $p < 0.01$), respectively (Tabela 2.4).

2.3 DISCUSSION

Potential habitat suitability modelling revealed that the spatial distribution of *An. cruzii* is correlated with areas of ombrophilous dense forest that are present in the mountainous areas of the Vale do Ribeira (Figura 2.2B, 2.3B), whereas *An. bellator* spatial distribution was determined by high Bio5 and Bio12 on the coastal low lands where the vegetation is the Atlantic Coast Restinga forest (Figura 2.2A, 2.3A). Moreover, *An. marajoara* spatial distribution is correlated with high Bio5 and open areas of the Restinga or deforested lands associated with transformations of the natural ecosystem (Figura 2.2C, 2.3C) (Forattini et al., 1995; Conn et al., 2002). Although differences among the values of Bio5 and Bio12 were low, they were observed to be significant factors for distribution for *An. bellator* and *An. marajoara* (Tabela 2.3). These findings may be related to the coarse 900 meter-spatial resolution of these variables for both the target mosquito population and study area. Also the significance of Bio5 and Bio12 may

represent an indirect association with distribution of both species.

The AUC is not the best validation of potential habitat suitability maps when several niche factors of a species are poorly known. However, even considering the biases on the AUC related to the extrapolation of the data beyond a clustered sampled site (Lobo et al., 2008), the potential habitat suitability maps obtained are consistent with both ecological characteristics (Veloso, 1958; Forattini et al., 1999; Marrelli et al., 2007) and registers of the presence of *An. bellator*, *An. cruzii* and *An. marajoara* (Forattini and Massad, 1998) in Vale do Ribeira. The potential habitat suitability maps were also validated by the probability values higher than 80% for species control sites (Figura 2.3, Tabela 2.1).

Results of the present study were to associate mosquito potential distribution with malaria cases; these results are of further interest because they show that the spatial distribution of *An. cruzii* and *An. bellator* do not overlap at a larger geographical scale. *An. cruzii* is present on the hills, whereas *An. bellator* is present along the coastal shore of Vale do Ribeira. This lack of range overlap could be the result of either chance distribution or the competitive exclusion described by Gause (1934) because several unknown ecologic niche dimensions determine their actual distribution. Additionally, the potential habitat suitability for *An. marajoara* indicates that this species has a broader potential spatial distribution than that hypothesized by Wilkerson et al. (1995) in the Vale do Ribeira. As a result, further studies will be necessary to validate the prediction distribution models generated herein. Finally, it would be important to sample mosquitoes in areas with a high probability of the presence of the species that have never been sampled.

Results of the Poisson regression suggest that the presence of *An. bellator* is not significantly associated with malaria (Tabela 2.4). In contrast, this disease is significantly associated with *An. marajoara* and the presence of both *An. marajoara* and *An. cruzii* (Tabela 2.4). These results are partially in accordance with

the hypothesis of Curado et al. (2006) and Duarte et al. (2008), which suggest that in areas of the Atlantic Forest, humans can become infected with human *Plasmodium* when they invade the natural foci of *An. cruzii* and the *Alouatta* spp. of monkeys, which serve as the reservoirs of *Plasmodium* sp.. Moreover, Curado et al. (2006) performed enzyme immunosorbent assay tests and found a prevalence of antibodies against *P. vivax* in 63 (35%) and 155 (29.7%) humans from Parque Estadual Intervales and Parque Estadual do Alto do Ribeira, respectively. Interestingly, Curado et al. (2006) showed that the majority of the captured mosquitoes were *An. cruzii* in both areas, which are in the municipality of Iporanga. The spatial distribution modelling analyses indicated that the potential habitat suitability for this species was 95% in this municipality. However, the large and significant association between *An. marajoara* spatial distributions with cases of malaria was not in accordance with the hypothesis of Curado et al. (2006) and Duarte et al. (2008). This contradictory result might have occurred because these authors had not done their research in the municipality of Sete Barras, which had 40% of all autochthonous malaria cases occurring in Vale do Ribeira between 1996-2000 (Figura 2.2D). This underscores the need to sample mosquito assemblages from this municipality.

In considering the results obtained in the present study and the association between malaria cases and the potential spatial distribution of *An. marajoara*, we hypothesize that *An. marajoara* seems to be involved in the dynamics of malarial transmission in areas of the Vale do Ribeira. Cohuet et al. (2010) hypothesized that *Plasmodium* exerts selective pressure on its vectors in order to increase its own dissemination - shaping the vector to its own benefit. In this sense, it is probable that *Plasmodium* species is circulating in the Vale do Ribeira by keeping a sylvatic cycle that involves *Anopheles* (*Kerteszia*) mosquitoes and *Alouatta* monkeys as reservoirs and occasionally humans, as well as a cycle that involves humans and mosquito species of the subgenus *Nyssorhynchus* (e.g., *An. marajo-*

ara). This species was demonstrated to be a competent vector in Vale do Ribeira in the 1940s (Schiavi, 1945). Furthermore, Conn et al. (2002) showed that *An. marajoara* from Macapá became the primary vector of *Plasmodium* because of the increasing habitation of this species in non-forested and marshy lands used for agriculture, combined with the intensive human migration of infected individuals from other locations in the Amazon where transmissions were endemic.

In Vale do Ribeira, deforestation and changes in the land use may have affected the dynamics of malarial transmission, leading to a local succession of vector species of the subgenus *Kerteszia* and of the subgenus *Nyssorhynchus* (e.g., *An. marajoara*). In addition, adult populations of *An. albitarsis* l.s. are abundant and dominant in irrigated rice fields (Forattini et al., 1995), showing that these human-modified environments can create ecological conditions that favour species of the *Nyssorhynchus* subgenus that are vectors of *Plasmodium*.

As landscape structure is an important determinant for the dynamics of infectious diseases, man-made modifications can create ecological and microclimate conditions that favour some mosquito vector species and also the pathogens they transmit (Castro et al., 2006; Olson et al., 2010). Vittor et al. (2006) showed that deforestation increased the rate of contact between *An. darlingi* and humans in the Peruvian Amazon, thereby increasing the incidence of malaria in the region. Similarly, Castro et al. (2006) demonstrated that the early stages of frontier settlement are dominated by environmental risks of malarial transmission. Olson et al. (2010) showed that 4.3% of changes in the natural environment caused by deforestation were associated with a 48% increase in malarial incidence in the municipality of Mâncio Lima, state of Acre. Similarly, in the Vale do Ribeira, ecological conditions that favour the presence of habitats for *An. marajoara*, combined with the migration of individuals infected with human *Plasmodium*, might change the current status of low and dispersed malarial transmission in that region.

The results of the present study suggest that malarial transmission will continue and may become exacerbated if the proportion of natural forest remnants is reduced in the Atlantic Forest.

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Capítulo 3

Resource availability-heterogeneity and mosquito biodiversity in the Parque Estadual da Ilha do Cardoso

Neste capítulo, a ser submetido para publicação¹, estudou-se o efeito da heterogeneidade espacial dos habitats na riqueza e dominância de mosquitos em gradiente de vegetação preservada e biodiversa no Parque Estadual da Ilha do Cardoso, Mata Atlântica. Como resultado principal, a heterogeneidade dos habitats foi positivamente correlacionada com maior riqueza e equitabilidade na comunidade de mosquitos. A conclusão é que o efeito da heterogeneidade espacial aumenta a biodiversidade de mosquitos e pode manter níveis baixos de transmissão de parasitos, sendo este um serviço ecossistêmico de regulação de doenças infecciosas emergentes aos seres humanos.

Abstract. Mosquitoes evolved complex interactions with their environment. In tropical rain forests, mosquito biodiversity is high. However, determinants of mosquito assemblage richness and evenness-dominance are poorly known in Atlantic tropical rain Forest. The objective of the present study was to assess the effect of quantity and heterogeneity of forest resources on the biodiversity of mosquito assemblages in a primary tropical rain forest. Parque Estadual da Ilha do Cardoso was the locality employed as field station. Collections were undertaken in a gradient of vegetation, varying from 1-4 m height scrub vegetation to 25-30 m height ombrophilous dense forest. Three functional groups were determined based on general mosquito larval habitats: water accumulated on ground (ground), water accumulated on plants (phytothelmata, except bromeliads) and water accumulated on bromeliad tanks (bromeliad). Richness and evenness-dominance patterns in each functional group were quantitatively analyzed using hypothesis-testing, multi-model selection approach, and path analysis. As a result, pattern of ground mosquito richness was positively correlated with spatial heterogeneity of dry biomass litter. Discrepancy between dry biomass litter and vegetation structure, i.e., unexpected uncorrelated variables, was positively correlated with ground mosquito equitability. Vegetation structure was

¹to be submitted for publication in a peer-reviewed scientific journal.

positively correlated with both total abundance and richness of phytothelmata mosquito species. Quantity of bromeliad plants was correlated with bromeliad mosquito total abundance, but not with bromeliad mosquito richness. Phytothelmata mosquito dominance was associated with vegetation structure, but the dominant species were not vectors. Bromeliad plant quantity and heterogeneity was associated with dominant bromeliad species abundance. In addition, *Anopheles cruzii* abundance, a dominant species and the main vector of *Plasmodium* in Atlantic Forest, was associated with a homogeneous distribution of bromeliads. Overall, spatial resource heterogeneity, which can occur naturally in preserved tropical rain forests, may increase ground and phytothelmata mosquito richness, decreasing ground mosquito dominance and abundance of malaria-parasite vector *An. cruzii*.

Thoughtful and profound understanding of mosquito ecology can provide knowledge for elucidating the role of these insects in the dynamics of vector-borne diseases (Lounibos et al., 1985; Juliano, 1998; Kitching, 2001; Lounibos, 2002; Juliano and Lounibos, 2005; Blaustein and Chase, 2007; Yee and Juliano, 2007; Merrill, 2008; Juliano, 2009). Tropical forests are known to have the highest numbers of species on earth (Tokeshi, 1999; Hillebrand, 2004; Lewinsohn and Prado, 2005), including highly diverse mosquito assemblages (Shannon, 1931; Bates, 1949; Forattini 1962, 1965; Foley et al., 2007; Hutchings et al., 2011). The Atlantic Forest is a tropical biome with outstanding biodiversity in highly heterogeneous biogeographical sub-regions (Galindo-Leal and Câmara, 2003). The biogeographical sub-region of Serra do Mar is the best preserved and also one of the largest continuous area of Atlantic Forest (Ribeiro et al., 2009). This sub-region encompasses tropical evergreen and seasonally flooded coastal forests (Alves et al., 2010) and an abundant mosquito assemblage with many species (Forattini et al., 1978, 1986, 1993). Transformations of the environment, i.e., deforestation and changes in the land use modifying natural ecosystems into agriculture and open lands, had a great impact on mosquito diversity (Forattini et al., 1993). Consequently, species richness can decrease and mosquito total abundance can increase, leading to high risk of emerging vector dominance (Dorvillé, 1996; Forattini and Massad, 1998; Forattini, 1998). Potential emerging vectors are *Aedes scapularis*

(Rocio virus [ROCV]; Mitchell e Forattini, 1984; Mitchell et al., 1986; Forattini et al., 1995a; Laporta et al., 2012), *Aedes serratus* (Yellow Fever virus [YFV]; Laporta e Sallum, 2011), *Culex nigripalpus* (Saint Louis encephalitis virus [SLEV]; Lopes et al., 1979; Forattini et al. 1995b), and *Anopheles marajoara* (*Plasmodium* species; Forattini et al., 1995c; Laporta et al., 2011). It is noteworthy that *Anopheles cruzii*, the main *Plasmodium* vector in Atlantic Forest (Lutz, 1903; Coutinho et al., 1943; Rachou, 1946; Coutinho, 1947; Rachou e Ferreira, 1947; Pinotti et al., 1947), is dominant in preserved forest environments because of its larval development in bromeliads (Forattini et al., 1978, 1986; Marques et al., 2012).

Forest environments are heterogenous, providing spatially variable resource availability to the species (Hunter, 1990; Bell and Lechowicz, 1994; Tokeshi, 1999; Tews et al., 2004). This may lead to increasing richness and evenness in mosquito community. Both mosquito community richness and evenness are in general good because of the low risk of parasite transmission to humans (Schäfer et al., 2006; Beketov et al., 2010). The knowledge of mechanisms that drive mosquito biodiversity may help to manage Atlantic Forest ecosystems that are currently threatened by human impacts (Metzger et al., 2010). Here, quantity and heterogeneity of resources were assessed to determine patterns of richness and evenness-dominance in three functional groups of mosquito assemblages in a region of tropical rain forest (Parque Estadual da Ilha do Cardoso), Atlantic Forest.

3.1 MATERIALS AND METHODS

3.1.1 Study area

Field collections were conducted in the Parque Estadual da Ilha do Cardoso, Atlantic Forest, state of São Paulo, Brazil. Mosquitoes were collected along two 4-km and 2-km transects that were determined to assess mosquitoes from a vegetation gradient (Bernardi et al., 2005). Thirty collection sites were sampled, being one site every 200 meters (Figura 3.1C). Mosquito sampling covered two different forest physiognomies (Veloza et al., 1991). The forest physiognomies is comprised of a vegetation mosaic, herein divided into restinga scrub and arboreal restinga, and lowland dense ombrophilous forest. The restinga scrub and arboreal restinga physiognomies are characterized by elevation from 0 to 50 m and sandy soil, whereas dense ombrophilous forest is present at an elevation that vary from 50 to 100 m altitude and clayed soil (Alves et al., 2010). Along these transects, richness (15) and mean abundance (2 ind/10 km) of medium and large mammals are high (Galetti et al., 2009) which means host availability for the biting mosquito species.

3.1.2 Mosquito collections and identification

Mosquito collections were conducted using CDC-CO₂ (from 600 am to 1800 pm) and CDC-light traps (from 1800 pm to 600 am), completing 24-hour of sampling effort in each of the 30 collection sites. CDC was set 1.5 m above the ground. Mosquitoes were collected in the most favorable period of the year when the monthly mean temperature and total precipitation were approximately 28°C and 200 mm, respectively. Collections of nocturnal mosquitoes with CDC-light traps were conducted from 24th March to 6th April 2009, whereas the diurnal

collections were performed from 23th March to 4th April 2010. It was assumed that there was no difference in mosquito diversity between 2009 and 2010 because both collections were performed in the same seasonal period with similar mean temperature (29.4°-2009; 29.2°-2010) and total precipitation (71.8 mm-2009; 79.5 mm-2010) (Cananéia station; Source: <http://www.ciiagro.sp.gov.br/>). CDC-CO₂ traps were adapted to remote tropical areas, in where electricity and dry ice are unavailable. Consequently, a CDC with a CO₂ gas cylinder supplier was employed for collections as described by Laporta and Sallum (2011).

Field collected mosquitoes were maintained in recipients with silica. Species identification was based on external morphological characters using identification keys from published literature (Lane and Whitman, 1951; Lane, 1953; Galindo et al., 1954; Correa and Ramalho, 1956; Bram, 1967; Pecor et al., 1992; Harbach, 1994; Sallum and Forattini, 1996; Forattini, 2002; Sallum et al., 2009). Mosquito nomenclature adopted here was that proposed by Guimarães (1997).

3.1.3 Functional groups

The functional group approach was utilized for applying an ecological-based classification for mosquito species (Shannon, 1931; Bates, 1949; Forattini 1962, 1965). Mosquito species were sorted into three groups, depending on larval habitats. Species that colonize ground waters were included in group 1, whereas those that utilize phytothelmata, except bromeliads, formed group 2. Group 3 is comprised of species that use bromeliad phytothelmata as habitat.

3.1.4 Resource variables

Habitat characteristics were assessed for each collection site. Accordingly, four transects of 15-m lengths and 1-m widths were established around each CDC trap (Figura 3.1D). The following variables were estimated: number of terrestrial bromeliad plants, number of tree holes, terrain slope, litter biomass, and vegetation structure. Terrestrial bromeliad plants and tree holes were counted in 60 m² of each collection site, whereas 4 m² of litter biomass per collection site was dried and weighted in laboratory. Terrain slope was measured in the field with an inclinometer. Data from vegetation structure was obtained following the same procedure adopted by Pardini et al. (2005). Accordingly, foliage density, i.e., vertical length of plant leaves, was measured for every plant stretching an imaginary vertical column of each of the five forest strata 0–1, 1–5, 5–10, 10–15, >15 meters above the ground. The vertical length of plant leaves which stretched along an imaginary vertical column of 150 mm in diameter was measured by a laser rangefinder BoschTM DLE50, a tool intended for measuring distances with a maximum range of 50 m. A 4-meter pole was utilized to help establishing an imaginary column.

For the analyses, habitat characteristics were assessed as independent variables. Considering the habitat characteristics of each transect (Figura 3.1D), the mean and the variance of terrestrial bromeliad plants and dry litter biomass were estimated for each collection site. The mean and the variance of terrestrial bromeliad plants represented average quantity and resource heterogeneity of bromeliad phytotelmata oviposition and larval development sites. The mean and the variance of dry litter biomass were employed as indicative of average quantity and resource heterogeneity of ground oviposition, larval development and adult resting sites. The mean of terrain slope inclination was estimated for each collection site and represented the suitability for presence of oviposition sites on

Figura 3.1: A: South America and states of Brazil; B: The estuarine-lagoon Iguape-Cananéia-Paranaguá region in the southeastern coast of São Paulo state; C: Parque Estadual da Ilha do Cardoso; D: Collection site. (Source: INPE, 2007.)

the ground. The mean of foliage density was estimated for each stratum in each collection site. A principal component analysis (PCA) on the means of foliage density for all strata was conducted following Pardini et al. (2005). Vegetation structure was estimated for each collection site with the first axis of this PCA, and was used as indicative of heterogeneity of phytotelmata larval habitats.

3.1.5 Analysis

Ground mosquito evenness-dominance was estimated for each collection site employing species relative abundance values following a Poisson log-normal distribution (Bulmer, 1974; Engen et al., 2002). Hypothesis-driven models were proposed to explain the pattern of ground mosquito species richness and evenness-dominance. The best model was selected utilizing the Akaike Information Criteria (AIC), applying the small-sample unbiased correction, i.e., AICc (Anderson and Burnham, 2002).

Path analysis was utilized to explain phytotelmata and bromeliad mosquito richness and abundance patterns. Boxplots were utilized to see differences in abundance distribution of dominant phytotelmata and bromeliad mosquito species according to resource quantity and heterogeneity.

All analyses were run with the software R 2.12 (R Development Core Team, 2011).

3.2 RESULTS

Following 720 hours of sampling effort, a total of 11,660 specimens belonging to 73 mosquito species was identified. Ground (44), phytotelmata (15), and bromeliad (14) mosquito species are shown in Figura 3.2.

Larval habitats	Species	group no.
On the ground	<i>Aedes fulvus</i>	1
	<i>Aedes perventor</i>	
	<i>Psorophora albigena</i>	
	<i>Psorophora albipes</i>	
	<i>Aedes hastatus</i>	
	<i>Aedes oligopistus</i>	
	<i>Aedes scapularis</i>	
	<i>Aedes serratus</i>	
	<i>Psorophora ferox</i>	
	<i>Aedes albifasciatus</i>	
	<i>Psorophora saeva</i>	
	<i>Anopheles mediopunctatus</i>	
	<i>Culex akritos</i>	
	<i>Culex abonnenci</i>	
	<i>Culex aliciae</i>	
	<i>Culex alinkios</i>	
	<i>Culex dureti</i>	
	<i>Culex faurani</i>	2
	<i>Culex galvaoi</i>	
	<i>Culex misionensis</i>	
	<i>Culex mollis</i>	
	<i>Culex pedroi</i>	
	<i>Culex putumayensis</i>	
	<i>Culex rabelloi</i>	
	<i>Uranotaenia calosomata</i>	
	<i>Uranotaenia incognita</i>	
	<i>Uranotaenia mathesoni</i>	
	<i>Uranotaenia pallidoventer</i>	
	<i>Coquillettidia chrysonotum</i>	
	<i>Coquillettidia venezuelensis</i>	
	<i>Culex bidens</i>	
	<i>Culex declarator</i>	
	<i>Culex ribeirensis</i>	
	<i>Culex sachettae</i>	
	<i>Mansonia pseudotitillans</i>	
	<i>Uranotaenia geometrica</i>	
	<i>Anopheles maculipes</i>	
	<i>Culex aureonotatus</i>	
	<i>Culex inadmirabilis</i>	
	<i>Culex intricatus</i>	
	<i>Culex ocosa</i>	
	<i>Culex oedipus</i>	
	<i>Culex pilosus</i>	
	<i>Culex vaxus</i>	
Phytotelmata	<i>Limatus durhami</i>	3
	<i>Limatus flavisetosus</i>	
	<i>Runcomyia cerquerei</i>	
	<i>Runcomyia frontosa</i>	
	<i>Runcomyia humboldti</i>	
	<i>Sabethes soperi</i>	
	<i>Sabethes intermedius</i>	
	<i>Trichoprosopon pallidiventer</i>	
	<i>Wyeomyia aporonoma</i>	
	<i>Wyeomyia confusa</i>	
	<i>Wyeomyia occulta</i>	
	<i>Wyeomyia shannoni</i>	
	<i>Runcomyia reversa</i>	
	<i>Runcomyia theobaldi</i>	
Tank bromeliad	<i>Wyeomyia pertinens</i>	
	<i>Anopheles homunculus</i>	3
	<i>Culex inimitabilis fuscatus</i>	
	<i>Culex ocellatus</i>	
	<i>Wyeomyia mulhensi</i>	
	<i>Wyeomyia pallidoventer</i>	
	<i>Wyeomyia quasilingirostris</i>	
	<i>Wyeomyia theobaldi</i>	
	<i>Anopheles cruzii</i>	
	<i>Culex aphylactus</i>	
	<i>Culex microphyllus</i>	
	<i>Culex imitator</i>	
	<i>Culex neglectus</i>	
	<i>Anopheles bellator</i>	
	<i>Culex gairus</i>	

Figura 3.2: Mosquito functional groups based on larval habitats. (Source: Shannon, 1931; Bates, 1949; Forattini, 1962, 1965.)

Terrestrial bromeliad plants were found at highest frequencies in arboreal restinga where the open-canopy and the low-nutrient soil favor Bromeliaceae such as, *Quesnelia arvensis*, *Nidularium procerum*, *Nidularium innocentii*, *Catopsis berteroniana*, and *Vriesea altodaserrae*. A total of 2,003 terrestrial bromeliad plants were found for all collection sites. Higher values of biomass litter were found in dense ombrophilous forest. This possibly occurred because dense ombrophilous forest has tall canopy stratified into three layers (e.g., lower, canopy, and emergent) with abundant live vegetal biomass which, in turn, increase biomass litter on the forest ground (Alves et al., 2010). The most suitable values of terrain slope for oviposition sites on the ground were found in restinga scrub and arboreal restinga in where it was approximately 0°, whereas values of terrain slope were up to 22° in lowland dense ombrophilous forest (Tabela 3.1).

Vegetation structure showed variations through forest physiognomies. For example, vegetation structure varied for the 30 collection sites from -1.10 to 2.20 (i.e., first axis of the PCA; Tabela 3.1) showing the gradient of vegetation from restinga scrub (only herbaceous layer, one to five m-maximum height) and arboreal restinga (herbaceous and canopy layers, seven to 15 m-maximum height) to dense ombrophilous forest (herbaceous, lower, canopy, and emergent layers, 16 to 29 m-maximum height). The first axis of the PCA explained 61.4% of the total variability on the means of foliage density in all strata. Negative values were found for both restinga scrub and arboreal restinga which have higher foliage density in herbaceous layer (e.g., high levels of terrestrial bromeliad plants). Positive values represented ombrophilous dense forest in where canopy and emergent layers of tall trees are abundant, meaning higher heterogeneity of phytotelmata larval habitats (e.g., plant axils [*Heliconia velloziana*, *Calathea longibracteata*]; tree holes [*Psychotria nuda*, *Ficus* sp.], bamboo internodes [*Chusquea bambusoides*, *Merostachys* sp.], and bromeliads [*Vriesea* sp., *Aechmea* sp.]).

Tabela 3.1: Collection sites and independent variables, Ilha do Cardoso, Atlantic Forest, Mar-Abr 2009.

Sites	Number of terrestrial bromeliad plants: mean, variance (in 15 m ²)	Dry litter biomass: mean, variance (g m ⁻²)	Mean of terrain slope (°)	Vegetation structure (1° PCA axis)
1	25, 620	16.80, 13.65	3	-0.91
2	0, 0	12.20, 11.13	2	-0.65
3	10, 133	12.74, 6.68	2	-0.97
4	15, 544	12.31, 22.72	2	-0.91
5	29, 66	14.73, 0.32	2	-1.01
6	17, 107	16.34, 10.87	6	-1.02
7	12, 263	10.74, 5.59	4	-1.10
8	1, 2	11.78, 20.22	2	-0.55
9	13, 509	15.49, 23.71	2	-0.77
10	19, 238	14.54, 18.72	1	-0.89
11	4, 24	30.50, 53.62	4	-0.45
12	60, 116	25.17, 8.93	0	-0.32
13	40, 2	14.39, 22.23	2	-0.41
14	11, 12	11.91, 36.01	2	-0.50
15	39, 210	19.80, 39.44	1	0.13
16	23, 11	13.50, 19.10	2	-0.26
17	31, 523	11.64, 10.96	4	-0.55
18	43, 108	8.86, 1.58	1	-1.02
19	61, 62	14.69, 26.02	2	-0.31
20	20, 104	12.37, 2.59	1	-0.03
21	29, 121	13.93, 7.78	0	-0.40
22	1, 6	16.11, 31.86	0	0.28
23	0, 0	9.36, 10.19	0	1.59
24	0, 0	8.18, 4.46	2	1.94
25	0, 0	15.83, 6.43	1	2.09
26	0, 0	23.43, 34.54	6	1.26
27	0, 0	17.28, 13.30	4	1.04
28	0, 0	21.79, 18.02	10	2.18
29	0, 0	16.04, 15.11	11	0.69
30	0, 0	20.03, 8.25	7	1.84

Forest physiognomies in the collection sites: Sites 1-10: restinga scrub; Sites 11-20: arboreal restinga; Sites 21-30: lowland ombrophilous dense forest.

Ground mosquito were richer and more abundant than phytothelmata and bromeliad mosquito functional groups (Tabela 3.2). *Coquilleltidia chrysonotum* was the most dominant species in ground mosquito, whereas dominant species varied in phytothelmata and bromeliad mosquito functional groups (Tabela 3.2).

Tabela 3.2: Functional groups and their richness, total abundance, evenness, and dominant species.

Site	Functional group 1 ground mosquitoes				Functional group 2 phytothelmata mosquitoes			Functional group 3 bromeliad mosquitoes		
	Richness	Evenness	Abundance	Dominant ^a species	Richness	Abundance	Dominant ^b species	Richness	Abundance	Dominant ^c species
1	9	2.37	406	1	0	0	-	3	12	1
2	7	1.33	123	2	0	0	-	3	3	-
3	11	3.27	222	2	0	0	-	5	14	2
4	10	3.10	213	2	0	0	-	7	23	3
5	10	3.55	582	2	0	0	-	2	6	4
6	14	3.67	632	2	0	0	-	6	39	5
7	8	3.34	226	2	0	0	-	4	16	5
8	8	3.09	96	2	0	0	-	1	1	-
9	7	2.29	131	2	0	0	-	2	3	5
10	8	3.45	154	2	0	0	-	5	13	5
11	22	2.77	627	2	3	14	1	9	35	3
12	17	2.15	283	2	2	2	-	7	69	3
13	19	3.09	327	2	1	7	-	9	52	3
14	13	3.2	386	2	2	4	-	8	20	3
15	14	2.78	250	2	1	3	-	6	15	4
16	12	3.33	555	2	2	2	-	6	45	4
17	14	3.31	433	2	1	1	-	6	66	3
18	6	2.96	572	2	3	5	1	6	45	2
19	14	3.61	2,452	1	8	12	1	6	154	2
20	12	2.80	254	2	7	34	1	5	66	3-2
21	15	3.39	522	2	7	10	2	5	20	6
22	15	1.88	144	2	2	4	-	4	14	4
23	13	3.07	159	2	4	9	3	5	19	4
24	9	1.80	155	2	5	15	3	2	13	4
25	9	2.36	167	2	5	34	3	3	20	4
26	8	3.30	93	2	8	21	3	4	11	4
27	7	3.34	119	2	8	18	3	2	10	4
28	7	3.01	92	2	5	10	3	6	52	4
29	4	1.88	49	2	2	2	-	6	54	4
30	3	3.67	89	2	2	4	3	2	26	4

Forest physiognomies in the collection sites: Sites 1-10: restinga scrub; Sites 11-20: arboreal restinga; Sites 21-30: lowland ombrophilous dense forest.

^a: 1, *Aedes scapularis*; 2, *Coquilleltidia chrysonotum*.

^b: 1, *Runchomyia reversa*; 2, *Limatus flavisetosus*; 3, *Limatus durhami*.

^c: 1, *Anopheles bellator*; 2, *Wyeomyia mulhensi*; 3, *Wyeomyia quaslongirostris*; 4, *Anopheles cruzii*; 5, *Culex imitator*; 6, *Wyeomyia pallidoventer*.

-: Only one species is present in the functional group. Therefore, a dominant species cannot be inferred.

The multi-model framework was employed with either a model supported by literature or proposed in the present work (Tabela 3.3). The best model that explained the pattern of ground mosquito richness was 4a (resource heterogeneity of dry biomass litter effect; $\beta_1 = 3.11$ [z-value = 3.79, $P < 0.001$], $\beta_2 = 0.14$ [z-value = 2.87, $P = 0.004$]). The best model that explained the pattern of ground mosquito evenness was 3b (interaction between dry biomass litter and vegetation structure effects; $\beta_1 = 2.05$ [z-value = 3.19, $P = 0.001$], $\beta_2 = 0.05$ [z-value = 2.95, $P = 0.003$]) (Tabela 3.3). The interaction between dry biomass litter and vegetation structure represented two situations, being as first expected correlated variables (non-discrepancy) and second unexpected uncorrelated variables (discrepancy), which was correlated with evenness in ground mosquito functional group (Figura 3.3).

Tabela 3.3: Multi-model framework with Gaussian linear models: patterns of richness (models 1a-4a) and evenness (models 1a-3a) in the functional group 1 of ground mosquitoes.

Number	Model	Description	Supported by
1a (Null model) AICc = 168.67	Richness = $\beta_0 + \beta_1 * \text{abundance}$	Abundance increases species richness by increasing mean equilibrium population size, thereby reducing the probability of local extinction	Gotelli and Colwell (2001); Yee and Juliano (2007)
2a (Terrain slope effect) AICc = 163.84	Richness = $\beta_0 + \beta_1 * \text{abundance} + \beta_2 * \text{mean of terrain slope}$	Species richness is higher in lowland larval habitats (in where permanent water pools are more common, what propitiates mature aquatic biota with more niche availability)	Schäfer et al. (2006); Eisen et al. (2008); Beketov et al. (2010)
3a (Dry biomass litter effect) AICc = 162.20	Richness = $\beta_0 + \beta_1 * \text{abundance} + \beta_2 * \text{mean of dry biomass litter}$	Coexistence among mosquito species that utilize habitats in the ground may increase in function of resource availability (litter biomass), which can especially be important for the species-rich subgenus <i>Melanoconion</i> of <i>Culex</i>	Pecor et al. (1992); Sallum and Forattini (1996)
4a (Resource heterogeneity of dry biomass litter effect) AICc = 159.26^a	Richness = $\beta_0 + \beta_1 * \text{abundance} + \beta_2 * \text{variance of dry biomass litter}$	Resource heterogeneity can modify biotic interactions in larval habitats, which may increase species richness on ground waters	Juliano (2009); This work
Number	Model	Description	Supported by
1b (Null model) AICc = 60.12	Evenness $\sim \text{Normal}(\bar{\mu}; \text{SD})$	Unspecified “random” forces generate variation that excludes species interactions	Gotelli and McGill (2006)
2b (Abundance effect) AICc = 57.61	Evenness = $\beta_0 + \beta_1 * \text{abundance}^b$	Increase in total abundance intensifies interspecific resource competition which unbalances relative abundances.	Juliano (1998); Tokeshi (1999)
3b (Interaction between dry litter biomass and vegetation structure effects ^c) AICc = 52.63^a	Evenness = $\beta_0 + \beta_1 * \text{abundance}^b + \beta_2 * \text{mean of dry biomass litter} * \text{vegetation structure}$	Litter biomass and vegetation structure represent resource availability for mosquito species that utilize, respectively, habitats in the ground and on vegetation strata. Considering that ground water species and phytothelmata species can utilize both resources (vegetation structure and biomass litter), interactive effects of such resources can occur, which may have an effect in evenness pattern	Hubbell (2001); This work

^a: Text in bold means the best model (ΔAICc among models > 2).

^b: Here, evenness has a non-linear power asymptotic relationship with abundance.

^c: This interaction represents two situations: first, expected correlated variables (non-discrepancy); second, unexpected uncorrelated variables (discrepancy; see Figura 3.3).

Figura 3.3: Evenness-dominance of ground mosquitoes in function of interaction between dry biomass litter and vegetation structure (**red linear function**). Discrepancy values mean that collections sites 11, 12, 23, 24 have a discordance of dry biomass litter compared to the observed vegetation structure. Non-discrepancy is generally expected because of the strong and positive correlation between biomass litter and vegetation structure (Alves et al, 2010). Here, this discrepancy was correlated with ground mosquito evenness.

Figura 3.4: Path analysis. A: V, vegetation structure is both directly and indirectly (through Ab_f , abundance) correlated with R_f , richness of phytotelmata mosquito functional group; B: B, the mean number of terrestrial bromeliad plants is correlated with Ab_b , abundance, that is correlated with R_b , richness of bromeliad mosquito functional group.

Path analysis showed that vegetation structure was correlated with both abundance and richness of phytothelmata mosquito, whereas terrestrial bromeliad plants were correlated with abundance of bromeliad mosquito functional group (Figura 3.4).

Figura 3.5: Effect of vegetation structure on phytothelmata dominant mosquito species. 1, *Runchomyia reversa*; 2, *Limatus durhami*.

Runchomyia reversa was dominant in restinga scrub, whereas *Limatus durhami* was dominant in lowland ombrophilous dense forest (Figura 3.5).

Anopheles cruzii was dominant in sites in where terrestrial bromeliads were in lower numbers of plants (Figura 3.6A). This species was dominant in sites in where spatial distribution of terrestrial bromeliad plants was homogeneous (i.e., small variance; Figura 3.6B).

Figura 3.6: Boxplot on bromeliad dominant mosquito species. 1, *Wyeomyia quasilongirostris*; 2, *Anopheles cruzii*; 3, *Wyeomyia mulhensi*; 4, *Culex imitator*. A: Effect of the mean of terrestrial bromeliad plants; B: Effect of the variance of terrestrial bromeliad plants

3.3 DISCUSSION

Here, we assessed quantity and heterogeneity of habitat determinants to correlate with biodiversity of three mosquito functional groups based on the main larval habitats: water accumulated on the ground, water accumulated in phytothelmata (except bromeliad), and water accumulated in bromeliads (Shannon, 1931; Bates, 1949; Forattini, 1962, 1965).

Heterogeneity of biomass litter was strongly and positively correlated with ground mosquito richness. Biomass litter represents resource availability, i.e., gross organic matter that will be decomposed in the presence of water providing oviposition sites to ground larval mosquito development (Clements, 1999). Species-rich subgenus *Melanoconion* of *Culex* is usually associated with high organic matter concentration in ground water larval habitats in Atlantic Forest (Sallum and Forattini, 1996). High variance of biomass litter in a local may provide several specific contexts that increase ground mosquito species richness, which is supported in the work of Juliano (2009).

Discrepancy between the quantity biomass litter and vegetation structure is not expected. More structured, ombrophilous dense forest has three to four plant leaf strata, that increase biomass litter. Less structured, restinga scrub and arboreal restinga have one to two plant leaf strata, leading to less biomass litter (Alves et al, 2010). Considering high levels of heterogeneity in ecosystems of Atlantic Forest (Silva and Casteleti, 2003; Ribeiro et al., 2009), discrepancy between the quantity biomass litter and vegetation structure may occur naturally caused by unknown complex interactions in vegetation-soil nutrient cycling. It is noteworthy that discrepancy was strongly and positively correlated with ground mosquito evenness. Considering the effect of metapopulation structure in community evenness (Hubbell, 2001), mixture of discrepancy and non-discrepancy sites can increase global spatial heterogeneity, providing heterogenous resource

availability which may decrease ground mosquito dominance locally.

Vegetation structure represents availability of phytothelmata larval habitat types. Ombrophilous dense forest can provide a full range of specific larval habitats, such as plant axils, bamboo internodes, tree holes and epiphytes (Forattini 1962, 1965; Clements, 1999). On the other hand, restinga scrub and arboreal restinga have generally one phytothelmata type, e.g., terrestrial bromeliad plants (Melo et al., 1991). Heterogeneity of phytothelmata larval habitats in ombrophilous dense forest may be the cause of increasing phytothelmata mosquito richness.

Terrestrial bromeliad plants are important larval habitats for mosquitoes (Clements, 1999; Kitching, 2001; Frank and Lounibos, 2009). Mosquito species richness is high where there are more terrestrial bromeliad plants because of the increase in mosquito abundance. This hypothesis was supported by Yee and Juliano (2007) who suggested that species abundances may determine variations on mosquito species richness because they are related with the decreased probability of local population extinction. Therefore, the number of bromeliads (resource availability) may determine bromeliad mosquito richness.

Exploitation of food resources in larval habitats is related to specialization of mosquito larval mouth parts, which present a degree of morphological adaptations (Harbach, 1977; Merritt et al., 1992). Across heterogeneous landscapes, presence of specific mosquito species is related to presence of specific larval habitat (Juliano, 2009). In the present work, mosquito vectors species, which are usually associated with larval habitats in rural landscapes (Forattini and Massad, 1998), were either not found (e.g., *Anopheles marajoara* and *Culex nigripalpus*) or collected showing low abundance and dominance (e.g., *Aedes scapularis*). In phytothelmata mosquito functional group, *Runchomyia reversa* is dominant in restinga, whereas *Limatus durhami* is dominant in forest, indicating coexistence mediated by partitioning of different larval habitats (Forattini, 1965). In bromeliad mosquito functional group, *Culex imitator* is dominant in restinga scrub,

while *Wyeomyia* (*Phonomyia*) species are dominant is arboreal restinga. *Anopheles cruzii* can be dominant in ombrophilous dense forest (Forattini et al., 1986) in where it frequently occurs in terrestrial bromeliad plants (Marques et al., 2012). In the present work, this species was locally dominant in sites having homogeneous distribution (i.e., less variance) of terrestrial bromeliad plants (see Figura 3.6B) in both ombrophilous dense forest and arboreal restinga (see Tabela 3.2). Therefore, *An. cruzii* dominance may be high in forest environments that have randomly distributed larval habitats.

Generally speaking, results of the present study indicate that mosquito diversity can be utilized for studying and monitoring biodiversity in Atlantic Forest because of three main reasons. First, ground and phytotelmata mosquito species richness is associated with local resource heterogeneity. Second, ground mosquito evenness was correlated with discrepancy between litter biomass quantity and vegetation structure which may be indirectly related with spatial heterogeneity of natural habitats. Third, intact tropical forests and its inherent spatial heterogeneity can negatively affect mosquito vector emergence, decreasing the probability of raising a dominant mosquito vector species. Further studies are necessary for including mosquitoes as indicators for conservation planning and future avenues could be the roles of mosquito biodiversity in heterogeneous/homogeneous and disturbed/undisturbed ecosystems.

3.4 REFERENCES

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Capítulo 4

Biodiversity effects on bromeliad malaria in the Parque Estadual da Ilha do Cardoso

O presente capítulo¹ é para demonstrar por meio de um novo modelo matemático de bromélia-malária que a biodiversidade pode diminuir o risco de transmissão de plasmódios aos humanos. Sob as condições hoje observadas no local de estudo (Parque Estadual da Ilha do Cardoso, Mata Atlântica), um surto de transmissão de parasitos da malária não seria possível. As condições observadas incluem a presença de espécies silvestres de mosquitos e de vertebrados. O modelo anterior (o clássico Ross-MacDonald) não considerava essas espécies e, portanto, é estimado maior risco de epidemia ($R_0 > 1$). A relação da biodiversidade como um serviço ecossistêmico para a regulação de doenças é discutida.

Abstract. The Parque Estadual da Ilha do Cardoso is a primary tropical forest, being protected by biodiversity conservation law in Atlantic Forest. Indigenous people and fishermen have the right to live in this region. Introduction of *Plasmodium* may occur in that region because of its location in Serra do Mar in where low-levels of endemic parasite transmission occur. Consequently, human population both from Parque Estadual da Ilha do Cardoso and summer tourists can be exposed to malaria-parasites. However, no malaria cases have been reported there in the last 30 years. Here, we provide a mathematical model that explains how biodiversity could hinder bromeliad malaria epidemic. As a result, absence of malaria was explained by our model, which takes into consideration some transmission blocking determinants that are present, such as the herein proposed ecological mechanisms: mosquito vector diffuse competition and dead-end parasite transmission. These mechanisms are ecosystem services that may be related to functional diversity and composition. These relationships between disease and diversity indicate that protecting biodiversity may reduce risk of emerging malaria in those areas.

Dynamics of malaria transmission are complex, encompassing *Anopheles*

¹it was submitted for publication to *PLoS Neglected Tropical Diseases*.

species vectors that can transmit *Plasmodium* protozoan parasites which cause human malarial infections in tropical and sub-tropical zones in Asia (Sinka et al., 2011), Africa (Sinka et al., 2010a), and Americas (Sinka et al., 2010b). The Global Malaria Eradication Program, launched in the middle of the last century, over-promised and under-delivered, mainly because of optimistic forecasts that had been based on an oversimplified view of transmission ecology (Fergusson et al., 2010). As a result, World Health Organization reported approximately 82 million confirmed malaria cases and 120 thousand malaria attributed deaths in 2010 (WHO, 2010). However, the interruption of malaria-parasite transmission worldwide is firmly back on the global health agenda in order to identify key knowledge gaps and to define the strategies (e.g., vector control and prophylaxis drugs) that will result in reducing the basic reproduction rate to less than 1 (Alonso et al., 2011). The basic reproduction rate, R_0 , is the expected number of human cases that would arise from each human case in a population, being a standard measure to achieve malaria elimination locally (The malERA, 2011).

Malaria is a global public health problem today, but became nevertheless a low-endemicity *Plasmodium* transmission disease in the Brazilian Atlantic Forest (Curado et al., 2006; Crispim et al., 2007; Marrelli et al., 2007; Duarte et al., 2008; Oliveira-Ferreira et al., 2010). Atlantic Forest is a world biodiversity hotspot (Galindo-Leal and Câmara, 2003). The most preserved and continuous forested area is in the biogeographical sub-region Serra do Mar, southeastern Brazil (Ribeiro et al., 2009). In that forested area where both mosquito and vertebrate richness are high, malaria is hypo-endemic (Oliveira-Ferreira et al., 2010) and mostly associated with *Kerteszia* species as vectors. Lutz (1903) suggested that transmission of *Plasmodium* among outdoor workers in Serra do Mar, Atlantic Forest, southeastern São Paulo State was caused by *Anopheles* (*Kerteszia*) *cruzei* mosquito. The larvae of this mosquito species depends on bromeliads for larval development and thus Downs and Pittendrigh (1946) and Smith (1952)

suggested the term bromeliad malaria for the transmission dynamics of malaria that involve *Kerteszia* mosquitoes as vectors. Epidemics of bromeliad malaria occurred with 5% incidence in the states of Santa Catarina and Paraná, southern Atlantic Forest, from 1944 until 1951 (Smith, 1952). Malaria epidemics mainly ceased because of a deliberate deforestation that eliminated 3,800 Km² of native forest (Reitz, 1983). The vector control strategy was focused on removing most mosquito larval habitats, such as bromeliads, and reducing adult resting places in forest (Smith, 1952).

Currently, bromeliad malaria persists with low-frequency and spatial-temporally dispersed cases (Oliveira-Ferreira et al., 2010) in natural foci where *An. cruzii* is a vector of human *Plasmodium vivax* and variants, and *P. malariae* (Curado et al., 2006; Duarte et al., 2008). In Atlantic Forest, autochthonous transmission mainly occur in the biogeographical sub-region of Serra do Mar (Couto et al., 2010). In this sub-region, topographically complex arrangements and high level of vegetation cover favor an outstanding fauna and flora diversity (Galetti et al., 2009; Alves et al., 2010). Ecological interactions among vector-*Plasmodium* and local biodiversity may drive to mechanisms that modulate malaria transmission. Natural ecosystems in Serra do Mar can offer a various range of habitats for mosquito species and thus it is expected both a high mosquito species richness and a equal species abundance. These two factors combined may decrease the number of infective bites, when several mosquito species are trying to feed on a defensive host (Edman and Kale, 1971; Pianka, 1974) and may decrease succesful bites for mosquito vector population. In another scenario, malaria-parasite transmission may be affected by abundance of non-competent hosts that divert mosquitoes from feeding on, and transmitting *Plasmodium* to humans (Saul, 2003), which represent a dilution effect of wild warm-blooded animals that act as dead-end hosts. Therefore, ecological interactions, namely mosquito vector diffuse competition and dead-end parasite transmission, are expected to have a negative impact

on the risk of bromeliad malaria emergence, which would explain low malaria endemicity in Atlantic Forest.

The impact of biodiversity on the emergence and transmission of infectious diseases is a new focus of conservation studies (Keesing et al., 2010). Some authors showed that when biodiversity declines, the risk of diseases that affect coral animals increases (Raymundo et al., 2009). Similarly, risk of Schistosomiasis (Jonhson et al., 2009), West Nile Fever (Swadle and Callos, 2008), Hantavirus disease (Suzán et al., 2009), Lyme disease (Keesing et al., 2009), and Malaria (Carlson et al., 2009) in humans is higher when biodiversity is negatively impacted by human activities. Relationship between decline of biodiversity and elevation of risk of a vector-borne disease may be caused by either change in host and vector abundances or modification in host, vector, and parasite behavior (Keesing et al., 2010). A negative correlation between human cases of vector-borne diseases and loose of biodiversity was proposed for tick-borne *Borrelia burgdorferi* infection that can cause Lyme disease in the United States (Ostfeld and Keesing 2000; Begon et al., 2006). Moreover, authors hypothesized that a dilution effect can be accomplished when the following ecological factors are present: 1) a vector is generalist regarding its feeding behavior; 2) a pathogen can be transmitted horizontally; 3) community has a variety of vertebrate species in which some are not a reservoir, whilst others are competent hosts to transmit a pathogen to a susceptible vector (Ostfeld and Keesing 2000; Begon et al., 2006). However, other mechanisms could also be involved to explain the decline in malaria-parasite transmission (Carlson et al., 2009; Meyrowitsch et al., 2011). For example, *Anopheles gambiae* s.s. abundance may decrease because of higher diversity of both predators and competitors of mosquito larvae in Africa (Carlson et al., 2009; Kirby and Lindsay,). In addition, interspecific competition could possibly affect *Anopheles darlingi* abundance when females have an overlapping biting-activity period and are trying to bite upon a host in Amazon region (Tadei

et al., 1998). Considering unimportance of predation (Juliano, 2009) and specialized resource partitioning (Harbach, 1977; Merritt et al., 1992) in bromeliads, biting-activity of *An. cruzii* could be affected by other mosquito species and may have a role in the dynamics of bromeliad malaria.

Deforestation as a *An. cruzii* control strategy is currently unacceptable, being a cause of controversy between public health administrators and conservationists (Smith, 1952; Reitz, 1983). Considering the current Atlantic Forest conservation program, and the urgent need for its biodiversity restoration (Galindo-Leal and Câmara, 2003), it would be important to understand interactions between dynamics of bromeliad malaria and both diversity of vertebrates and mosquito assemblage composition. The knowledge of ecological mechanisms that interacts in the dynamics of malaria-parasite transmission may support arguments against human occupation of natural protected areas, and thus favor those that defend biological conservation. Here, we provide a mathematical model that explains how biodiversity can hinder bromeliad malaria epidemic. The objectives of the present study were to: (i) assess mechanisms relative to abundances of both non-vector mosquito and non-host vertebrate species in the epidemiology of bromeliad malaria; and (ii) to propose a novel mathematical model with explicit mechanisms of mosquito vector diffuse competition and dead-end parasite transmission. The study was conducted in a protected area which is inhabited by an indigenous population and fishermen, and in where *An. cruzii* is present but no malaria cases have been reported in the last 30 years, in the biogeographical sub-region Serra do Mar of Atlantic Forest.

4.1 MATERIALS AND METHODS

4.1.1 Study area

The estuarine-lagoon Iguape-Cananéia-Paranaguá region is a coastal land situated between the Ribeira de Iguape River and the Atlantic Ocean in the southeastern coast of Brazil (Figura 4.1A, 4.1B). Three great islands stretch along a hundred kilometers from northeast to southwest, namely Comprida, Cananéia, and Cardoso (Suguiu and Petri, 1973). The Cardoso island, hereafter Parque Estadual da Ilha do Cardoso, is a São Paulo State Park of the Brazilian Atlantic Forest (Tabarelli et al., 2005).

The Parque Estadual da Ilha do Cardoso is separated from the mainland by the Ararapira Channel, which is a body of water that can be only 30 m-wide in some places and thus animals, such as muriqui *Brachyteles* (Ingberman et al., 2010), can go across. Common wild warm-blooded animals are quails *Odonophorus capueira*, toucans *Ramphastos*, guans *Penelope* and *Pipile jacutinga*, tinamous *Tinamus solitarius*, howler monkeys *Alouatta*, agoutis *Dasyprocta leporina*, and squirrels *Sciurus ingrami* (Bernardo, 2004). Vegetation types are in ecological succession, which forms an ascending gradient of forest structure from the coastal plain to the mountains. In the coastal plain there are dune, restinga scrub, and arboreal restinga vegetation over sandbanks. Tropical pluvial forest vegetation types are in lowlands, hillsides, and hilltops (Bernardi et al., 2005) (Figura 4.1C).

Descendents of European colonists previously occupied this protected area and the most important local activities were fishing and family farming (Almeida, 1946). Demographic density is approximately 500 people per 150 km² today, which would not cause relevant impact to local biodiversity. However, tourism has become one of the main incomes and thus thousands of tourists arrive every

summer in the fishing village of Marujá at the south (Figura 4.1C). In addition, indigenous peoples Guaraní Mbya have settled in the northwestern and have the right to hunt and to log in the forest for their own subsistence (Galetti, 2001).

4.1.2 Mathematical model of bromeliad malaria transmission

To estimate malaria-parasites transmission risk, the Ross-MacDonald model was employed for the two most populated human settlements, i.e., Guaraní and Marujá (Figura 4.1C). It was assumed that *An. cruzii* abundance was constant (Anderson and May, 1991; i.e., no ecological interactions), and thus the model was mathematically expressed (Keeling and Rohani, 2008) as follow:

$$\frac{dX_h}{dt} = -\frac{bT_{hm}X_hY_m}{N} + \gamma Y_h \quad (4.1)$$

$$\frac{dY_h}{dt} = \frac{bT_{hm}X_hY_m}{N} - \gamma Y_h \quad (4.2)$$

$$\frac{dX_m}{dt} = \mu Y_m - \frac{bT_{mh}X_mY_h}{N} \quad (4.3)$$

$$\frac{dY_m}{dt} = \frac{bT_{mh}X_mY_h}{N} - \mu Y_m \quad (4.4)$$

where X_h = suscetible humans; Y_h = infected humans; X_m = suscetible mosquitoes; Y_m = infected mosquitoes; $N = X_h + Y_h$; b = biting rate; T_{hm} = transmission probability from a biting infected mosquito to a human; T_{mh} = transmission probability from a infected human to a biting mosquito; γ = recovery rate by humans; and μ = mortality rate of vector mosquitoes.

To add ecological interactions, such as mosquito vector diffuse competition and dead-end parasite transmission, an alternative mathematical model was constructed utilizing the Ross-MacDonald model (Keeling and Rohani, 2008) as a base. Firstly, we considered that *An. cruzii* abundance was in function of parameters as follow: 1) mortality (μ); 2) biting rate (b); 3) conversion rate of

Figura 4.1: Study area. A: South America and Brazilian States; B: The estuarine-lagoon Iguape-Cananéia-Paranaguá region in the southeastern coast of Brazil; and C: Parque Estadual da Ilha do Cardoso. G, Guarani Mbya Indians settlement; and M, Marujá fishing village. [Source: Bird and mammal observations (Bernardo, 2004); Altitude and vegetation sampling (Bernardi et al., 2005) (see Supporting Information).]

one bite to *An. cruzii* adults (α); 4) biting tolerance exhibited by vertebrate animals and humans (h); 5) abundance of wild warm-blooded animals (B); and 6) abundance of non-vector mosquito species (C). Secondly, abundance of *An. cruzii* in equilibrium (X_m^*), i.e., in a given time and space, was thus dependent on the following equation:

$$X_m^* = \left(\frac{\alpha b}{\mu} - 1 \right) h(B + N) - C \quad (4.5)$$

in where the underlying assumption was that competition affects both *An. cruzii* and non-vector mosquito species and thus *An. cruzii* is not excluded by competition (Supporting Information). Therefore, our deterministic model was the following:

$$\frac{dX_h}{dt} = -\frac{bT_{hm}X_hY_m}{(B + N)\left(1 + \frac{1}{h}\frac{C+M}{B+N}\right)} + \gamma Y_h \quad (4.6)$$

$$\frac{dY_h}{dt} = \frac{bT_{hm}X_hY_m}{(B + N)\left(1 + \frac{1}{h}\frac{C+M}{B+N}\right)} - \gamma Y_h \quad (4.7)$$

$$\frac{dX_m}{dt} = \frac{\alpha b M}{1 + \frac{1}{h}\frac{C+M}{B+N}} - \mu X_m - \frac{bT_{mh}X_mY_h}{(B + N)\left(1 + \frac{1}{h}\frac{C+M}{B+N}\right)} \quad (4.8)$$

$$\frac{dY_m}{dt} = \frac{bT_{mh}X_mY_h}{(B + N)\left(1 + \frac{1}{h}\frac{C+M}{B+N}\right)} - \mu Y_m, \quad (4.9)$$

where $M = X_m + Y_m = X_m^*$.

To transform our model back to the Ross-MacDonald model was possible, being necessary to consider that wild warm-blooded animals did not interact with *An. cruzii* ($B = 0$); non-vector mosquito species did not interact with *An. cruzii* ($C = 0$); vertebrate animals and humans did tolerate mosquito bites ($h \rightarrow \infty$); and *An. cruzii* abundance was constant ($\alpha b = 0$). Finally, analysis was performed having inputs (parameters) to yield our model outputs, such as predictions of the basic reproduction number (R_0) under the effects of parameter

variations. The goal of the analysis was to examine the relationship between bromeliad malaria dynamics (synthesized by R_0 estimate) and ecological interactions hypotheses (formalized into model structure).

4.2 RESULTS

4.2.1 Mathematical model of malaria transmission

The indigenous Guarani Mbya settlement is inhabited by 150 indians in 2.8 Km², whereas 165 persons (fishermans and their families) live in Marujá fishing village in 0.8 Km² (N ; Figura 4.1C; Supporting Information). Birds *Odontophorus capueira*, *Ramphastos*, *Penelope*, *Pipile jacutinga*, *Tinamus solitarius*, and mammals *Alouatta guariba*, *Dasyprocta leporina*, *Sciurus ingrami* represent estimates of abundances of 172 and 47 wild warm-blooded animals (B) in indigenous Guarani Mbya settlement and Marujá fishing village, respectively (Tabela 4.1; Supporting Information). Estimates of abundances of mosquitoes are 300 for *An. cruzii* (X_m^*), 500 for *Ae. serratus* (C), 590 for *Ru. reversa* (C), and 2,550 for *Wy. quasilongirostris* (C) in Marujá fishing village and 1,514 for *An. cruzii* (X_m^*), 1,331 for *Ae. serratus* (C), 920 for *Ru. reversa* (C), 7,450 for *Li. durhami* (C), and 4,400 for *Wy. quasilongirostris* (C) in indigenous Guarani Mbya settlement (Tabela 4.1; Supporting Information).

Anopheles cruzii biting rate (b) is estimated as 0.5 in laboratory and field experiments (Tabela 4.1; Supporting Information). In laboratory, gonothrophic cycle length is four days and field experiment indicated gonothrophic discordance in natural populations (Tabela 4.1; Supporting Information). An estimate of 0.5 means that a mosquito bites every two days. *Anopheles cruzii* mortality rate (μ) is estimated in laboratory as 0.80 per day (Tabela 4.1; Supporting Information).

Therefore, parameter α which means how many new adults will be created from a single successful bite of *An. cruzii* is estimated as 5.5 in indigenous Guarani Mbya settlement and 3.1 in Marujá fishing village (Tabela 4.1; Supporting Information).

Probabilities of *Plasmodium* transmission and human recovery rate estimates are utilized here to represent malaria-parasite dynamics for a low-transmission area (Tabela 4.1; Supporting Information). Thus, mosquitoes transmit malaria-parasites to humans with 0.022 probability (T_{hm}), humans infect mosquitoes with 0.24 probability (T_{mh}), and human recovery rate (γ) is 0.0035 per day, which means that average duration of the infectious period is 286 days (Tabela 4.1; Supporting Information).

Parameter h which is host tolerance (the number of bites per day before host starts a defensive behavior) divided by mosquito biting rate is assumed as 20. This value represents that host defensive behavior will occur after the 10th bite per day (Tabela 4.1; Supporting Information).

The basic reproduction number (R_0) of the Ross-MacDonald model is as follow:

$$R_0 = \frac{b}{N} \sqrt{\frac{T_{hm} T_{mh} N X_m^*}{\gamma \mu}} \quad (4.10)$$

whilst basic reproduction number (R_0) to our model is the following:

$$R_0 = \frac{b}{(B + N) \left(1 + \frac{1}{h} \frac{C + X_m^*}{B + N}\right)} \sqrt{\frac{T_{hm} T_{mh} N X_m^*}{\gamma \mu}} = \frac{\mu}{\alpha(B + N)} \sqrt{\frac{T_{hm} T_{mh} N X_m^*}{\gamma \mu}} \quad (4.11)$$

where no bromeliad malaria cases is to be reported when $R_0 < 1$ and if it exceeds one ($R_0 > 1$) then the disease can invade and thus there will be an epidemic episode.

Tabela 4.1: Parameters, descriptions, estimates and references of the bromeliad malaria transmission model.

Parameter symbology	Description	Estimates	Reference
Human population size (N)	Total number of inhabitants in indigenous Guarani Mbya settlement and in Marujá fishing village	150 and 165, respectively	Supporting Information
Abundance of wild warm-blooded animals (B)	Estimates of abundance of avian and mammalian species in indigenous Guarani Mbya settlement and in Marujá fishing village	172 and 47, respectively	(Bernardo, 2004), Supporting Information
Abundance of non-vector mosquito species (C)	Estimates of abundance of non-vector mosquito species ^a in indigenous Guarani Mbya settlement and in Marujá fishing village	14,101 and 3,640, respectively	Supporting Information
Abundance of <i>Anopheles cruzii</i> (X_m^*)	Estimates of abundance of <i>An. cruzii</i> ^b in indigenous Guarani Mbya settlement and in Marujá fishing village	1,514 and 300, respectively	Supporting Information
<i>Anopheles cruzii</i> biting rate (b)	Biting rate of each <i>An. cruzii</i> female upon a given host per day	0.50	(Santos, 2001), Supporting Information
<i>Anopheles cruzii</i> mortality rate (μ)	Mortality rate of <i>An. cruzii</i> female population per day	0.80	(Santos, 2001), Supporting Information
<i>Anopheles cruzii</i> conversion rate (α)	Conversion rate of a successful bite upon a host to the number of emerging females in indigenous Guarani Mbya settlement and in Marujá fishing village	5.5 and 3.1, respectively	Equation 7, Supporting Information
Probability of <i>Plasmodium</i> transmission from <i>Anopheles cruzii</i> to humans (T_{hm})	Probability of <i>Plasmodium</i> transmission from <i>An. cruzii</i> to humans in low-endemicity malaria transmission dynamics	0.022	(Chitnis et al., 2008)
Probability of <i>Plasmodium</i> transmission from humans to <i>Anopheles cruzii</i> (T_{mh})	Probability of <i>Plasmodium</i> transmission from humans to <i>Anopheles cruzii</i> in low-endemicity malaria transmission dynamics	0.24	(Chitnis et al., 2008)
Human recovery rate (γ)	Daily human recovery rate, which can be understood as the average duration of the infectious period	0.0035 (286 days)	(Chitnis et al., 2008)
Host tolerance (h)	Number of bites per day before a host starts a defensive behavior divided by <i>An. cruzii</i> biting rate (0.5)	20, i.e., host defensive behavior occur after the 10 th bite in a given day	Supporting Information

^a: *Aedes serratus*, *Limatus durhami*, *Runchomyia reversa* and *Wyeomyia quaslongirostris*.

^b: *Anopheles cruzii* is the primary vector of bromeliad malaria *P. vivax* and *P. malariae* parasites (Curado et al., 2006).

Utilizing the R_0 estimate relative to Ross-MacDonald model (eq. 4.10), indigenous Guarani Mbya settlement has R_0 equal to 2.18 and in Marujá fishing village has R_0 equal to 0.93. Considering our model (eq. 4.11), the R_0 is 0.30 for indigenous Guarani Mbya settlement and 0.39 for Marujá fishing village. If abundances of both non-vector mosquito and non-host vertebrate species have approximately 80% and 70% reduction, respectively, then critical threshold level ($R_0 = 1$) is exceeded in indigenous Guarani Mbya settlement (Figura 4.2). In agreement, approximately 50% reduction in abundance of non-vector mosquito species can cause bromeliad malaria invasion ($R_0 > 1$) in Marujá fishing village (Figura 4.3). However, any reduction in abundance of non-host vertebrate species cannot cause the disease invasion ($R_0 > 1$) in Marujá fishing village (Figura 4.4). In addition, approximately 50% increase in human population can exceed the critical threshold level ($R_0 = 1$) in Marujá fishing village (Figura 4.4).

Finally, estimates of R_0 are sensitive to variations of the host tolerance (h) to indigenous Guarani Mbya settlement and Marujá fishing village. In qualitative terms, however, the role of studied mechanisms (i.e., mosquito vector diffuse competition and dead-end parasite transmission) in the bromeliad malaria dynamics remained similar (Supporting information).

4.3 DISCUSSION

The estimated $R_0 = 2.18$ obtained by the Ross-MacDonald model to indigenous Guarani Mbya settlement suggests that malaria-parasite transmission may occur in that region. However, no malaria cases have been reported there in the last 30 years. Absence of malaria can be also explained by our model, which takes into consideration some transmission blocking determinants that are present, such as the herein proposed ecological mechanisms (i.e., mosquito vector

Figure 4.2: Indigenous Guarani Mbya settlement. Increase in abundance of non-vector mosquito species and in abundance of wild warm-blooded animals is correlated with decrease in the risk of bromeliad malaria-parasite transmission. Reduction in abundance of wild warm-blooded animals (blue dashed arrow) and in abundance of non-vector mosquito species (red dashed arrow) can exceed the critical threshold level ($R_0 = 1$). The red circle is R_0 estimate of our model (0.3; eq. 4.11). The black isocline represents bromeliad malaria transmission threshold ($R_0 = 1$). Color legend shows a range of R_0 values from 0.00 to 1.40.

diffuse competition and dead-end parasite transmission) provided by high levels of biodiversity in the locality. These relationships between disease and diversity indicate that protecting biodiversity may reduce risk of emerging malaria in those areas.

Mathematical model proposed herein represents parasite transmission among four disease compartments that play specific roles into the dynamics of bromeliad malaria transmission in Atlantic Forest. The first compartment is susceptible hu-

Figura 4.3: Diffuse competition in Marujá fishing village. Increase in abundance of non-vector mosquito species is linearly correlated with decrease in the risk of bromeliad malaria-parasite transmission. Reduction in abundance of non-vector mosquito species (red dashed arrow) can exceed the critical threshold level ($R_0 = 1$). The red circle is R_0 estimate of our model (0.39; eq. 4.11). The black isoline represents bromeliad malaria transmission threshold ($R_0 = 1$). Color legend shows a range of R_0 values from 0.00 to 1.50.

man populations in the Guarani and Marujá settlements, which are in constant demographic dynamics. Considering that few humans are allowed to live within boundaries of Parque Estadual da Ilha do Cardoso (PEIC), human population size is approximately constant. However, malaria could emerge in this area because of its location in Serra do Mar in where low-levels of endemic parasite transmission occur (Curado et al., 2006; Duarte et al., 2008). Moreover, it is also plausible to assume that introduction of *Plasmodium* may occur in that re-

Figura 4.4: Dilution effect in Marujá fishing village. Increase in abundance of wild warm-blooded animals is quadratically correlated with decrease in the risk of bromeliad malaria-parasite transmission. Reduction in abundance of wild warm-blooded animals (red dashed arrow) does not exceed the critical threshold level ($R_0 = 1$). However, increase in human population size (blue dashed arrow) can exceed the critical threshold level ($R_0 = 1$). The red circle is R_0 estimate of our model (0.39; eq. 4.11). The black isoline represents bromeliad malaria transmission threshold ($R_0 = 1$). Color legend shows a range of R_0 values from 0.00 to 1.40.

gion because of the high number of humans that visit Vale do Ribeira during the summer, some are from endemic areas. Consequently, human population both from Parque Estadual da Ilha do Cardoso and summer tourists can be exposed to malaria-parasites. Human risk of *Plasmodium* infection in PEIC depends on *An. cruzii* biting rate (Santos, 2001) and probability of an infective bite (Chitnis et al., 2008), leading a proportion of susceptible humans to a second compartment,

which include infected humans. For this simple vector-human malaria-parasite transmission, however, ecological interactions can be added for a more realistic scenario of bromeliad malaria transmission. Other mosquito species (Supporting Information) and a diversified vertebrate community (Galetti et al., 2009; Bernardo, 2004) are intermixed and competing for food and spatial resources with mosquito vectors and humans. Thus, human malaria transmission may become difficult because of the increase in abundance of both non-vector mosquitoes and vertebrate animals. Infectious humans can recover, becoming again susceptible for malaria-parasites infection. This assumption was based on the lack of human cross-immunity against several *Plasmodium* sp that circulate in Atlantic Forest. Until infectious humans are not yet cured from infection, they can infect *An. cruzii* when blood-feeding (Santos, 2001) with a given probability of infection (Chitnis et al., 2008) in the third compartment, and thus leading a proportion of susceptible to infectious *An. cruzii* in the forth compartment. *Anopheles cruzii* was assumed to be in equilibrium, in which its mortality rate (Santos, 2001) was assumed in average to be smaller than its natality rate because of its high abundance in forested areas of Atlantic Forest (Smith, 1952; Marrelli et al., 2007). *Anopheles cruzii* natality rate was positively influenced by successful bites upon animals and humans, whereas it was negatively influenced by total abundance of non-vector mosquito species. Biting success was expressed by the average period of free biting until occurrence of host defensive behavior (i.e., the h parameter in Tabela 4.1; Supporting Information). For the human-vector malaria-parasite transmission, non-vector mosquito species decrease risk of human-vector contact, whereas increasing in number of animals and humans may increase vector abundance.

Effect of total abundance of non-vector mosquito species may have a significant negative impact on malaria-parasite transmission (Figura 4.2, 4.3). The cause of such effect is an apparent competition mediated by host-behavior that

can be defensive, decreasing risk of mosquito bites. Human hosts, for example, may react against mosquitoes. Animals also present defensive behavior (Edman and Kale, 1971). This was observed for blacklegged ticks which are vectors of the Lyme disease-parasite and can blood-feed upon a variety of mammals. However, attempts to blood-feed that are undertaken on very defensive hosts, such as opossums and squirrels, approximately kill 90% of these ticks (Keesing et al., 2009). In the present work, four mosquito species were selected to represent a mechanism of host-competition for *An. cruzii*. Each of these species can have a biting period overlapping with *An. cruzii* and total overlaps could then lead to the diffuse competition (Pianka, 1974) when many mosquito species are competing for the same host-blood. Considering that *An. cruzii* is embedded in a species-rich mosquito assemblage (Supporting Information), mosquito vector diffuse competition may be even more structured than results shown in Figura 4.2 and 4.3. Greater diversity increases the probability that an ecosystem has functional redundancy by containing other mosquito species that are capable of functionally replacing the non-vector species, driving to unsuccessful *An. cruzii* bites, which is supported by the insurance hypothesis (McCann, 2000). This is therefore related with an ecosystem service provided by species-rich mosquito assemblages that decreases malaria-parasite transmission in Atlantic Forest. In addition, the effect of diffuse competition may be considered as a control strategy that affects *Anopheles* vectors that do not rest or feed indoors and are therefore not susceptible to current tools (e.g., insecticide treated bednets) (Alonso et al., 2011). Reservations about the importance of this mechanism remain, however, because of the evolutionary forces exerted by the parasite that may alter vector capacity and competence parameters, when it is infected, in order to increase parasite own dissemination (Cohuet et al., 2010).

Wild warm-blooded animals can decrease *Plasmodium* transmission (Figura 4.2). These animals act as dead-end hosts and thus decreasing the risk of infec-

tive bites to humans, which can be an indirect method for malaria-control (i.e., zooprophylaxis) (Saul, 2003). The major concept of zooprophylaxis is to employ mammals as a barrier to stop mosquito carrying *Plasmodium* parasites from feeding upon humans (Fergusson et al., 2010; Saul, 2003). Zooprophylaxis may represent a dilution mechanism present in natural ecosystems with high abundance of wild warm-blooded species. This so-called dilution effect mechanism (Ostfeld and Keesing, 2000; Begon, 2006) was observed by Swadle and Calos (2008), Johnson et al. (2009), and Suzán et al. (2009) for West Nile fever, Schistosomiasis and Hantavirus disease, respectively. In communities in which *Schistosoma mansoni* circulates, there was a relationship between increasing host diversity and decreasing disease prevalence in an experimental study (Johnson et al., 2009). In another experimental study, decrease in host diversity was the main cause associated with increasing in hantavirus host abundance and infection (Suzán et al., 2009). During the West Nile virus (WNV) epidemics, 1998-2002, Swadle and Calos (2008) observed lower incidence of human WNV in eastern United States counties that have greater avian diversity. These are evidences supporting the dilution effect associated with high biodiversity (Ostfeld and Keesing, 2000; Begon, 2006). However, a low-level abundance of wild-warm blooded animals could lead to a neutral situation in which dilution effect is not important (Begon, 2006). Hunting activities that are allowed for traditional human communities in natural protected conservation units (e.g., Parque Estadual da Ilha do Cardoso) may cause empty forest effects (Galetti, 2001). Such effects are lower levels of vertebrate abundance compared to higher levels of vegetation and invertebrate abundances. In this situation, remaining wild warm-blooded animals can serve as blood sources for biting mosquito species, increasing vector population and may increase *Plasmodium* dissemination (Saul, 2003), which can be seen by a uni-modal relationship between abundance of non-hosts and critical threshold level ($R_0 = 1$) in Figura 4.4. Therefore, dilution effect of malaria-parasites may

occur in areas with high abundances of wild warm-blooded animals in Atlantic Forest.

Mosquito vector diffuse competition and dead-end parasite transmission are ecosystem services that are related to functional diversity (i.e., number of functional groups) and functional composition (i.e., which functional groups) may have large impacts in bromeliad malaria dynamics, which is supported in the work by Tilman et al. (1997). One question still remains : why was there a bromeliad malaria epidemic in the past, but not recently? *Anopheles cruzii* was found *Plasmodium*-infected in the intra-domicile during epidemics in the cities of Blumenau, Brusque, Joinville, and Florianópolis, in 1940 and 1950 decades (Smith, 1952). This suggests that people that struggle malaria-burden in those days live nearby forest in where bromeliads are larval habitats for *Kerteszia* mosquitoes. Although disease-control ecosystem services related with forest higher biodiversity were possibly present, other determinants may have been more important for increasing malaria-parasite transmission. One determinant might be the high increase in human susceptible population in those cities in a short period of time, reaching 800,000 human hosts (Smith, 1952). A second determinant might be the European ancestry of those populations that were removing native forest for agriculture and cattle farms, but living nearby jungle, what might have increased risk of humans to infective bites. Currently, heavily populated cities are distant from forested areas, and thus risk of bromeliad-malaria epidemics is low. However, recent malaria epidemics may have occurred because of similar ecological determinants present in highlands of Atlantic Forest in Espírito Santo State (Crispim, 2007) and in the southern border of of São Paulo municipality (CVE, 2006). In the latter region, a large contingent of human populations of Marsilac district have invaded natural forest reserve of Serra do Mar to construct their own houses. If human settlements in close contact with forest are represented by very small populations, such as in Parque Estadual da Ilha do Cardoso, then risk

of bromeliad malaria epidemics can be low in Atlantic Forest. Ecosystems should therefore be managed to sustain an equilibrium between high levels of biodiversity and an over-encroachment of human populations in order to achieve both desirable targets of biological conservation and maintenance of bromeliad-malaria low-endemic status in Atlantic Forest. Overall, understanding the effects of ecological mechanisms in this disease dynamics should be considered in public health and safety plans to manage the remaining forest and its local human populations.

4.4 SUPPORTING INFORMATION

Supporting Information was intended to better describe Materials and Methods section regarding estimates of input parameters which were utilized in the mathematical model of bromeliad malaria transmission.

4.4.1 Human population size (N)

The management plan of Parque Estadual da Ilha do Cardoso (Instituto Florestal/SP, 1998) was utilized to estimate population size in indigenous Guarani Mbya settlement and Marujá fishing village. Utilizing Aerophotogrametric images (Figura 4.5), it was possible to identify the geographical localities in where inhabitants logged to build houses or to cultivate crops. Indigenous Guarani Mbya settlement is in the northwestern, where approximately 150 people live today (Bernardo, 2004). They can log natural vegetation on lowlands to use smallscale agriculture and hunt animals in slopes of the primary tropical forest (Figura 4.5). Marujá fishing village occur on the margins of the southern coastal plain (Figura 4.5) in where 165 inhabitants live by fishing and tourism (Instituto Florestal/SP, 1998).

4.4.2 Abundance of wild warm-blooded animals (B)

Christine Bernardo and its advisor Dr. Galetti estimated the density of birds and mammals in Parque Estadual da Ilha do Cardoso (Tabela 4.1; Bernardo, 2004). Animal observations were performed up to 273 Km of transects in 13 trails throughout the region (Figura 4.6, Figura 4.7; Bernardo, 2004). Bird and mammal species density (Bernardo, 2004) was multiplied by the area of indigenous Guarani Mbya settlement (2.8 Km²; Figura 4.5) and Marujá fishing village (0.8 Km²; Figura 4.5). As a result, estimates of abundance of bird and mammal species were 172 and 47, respectively (Tabela 4.2).

4.4.3 Abundance of non-vector mosquito species (C) and *Anopheles cruzii* (X_m^*)

Mosquito species that have overlapping period of biting activity were collected employing CDC-CO₂ traps (Laporta and Sallum, 2011; ANEXO) from 600 am to 1800 pm in altitudinal and vegetational succession gradients. Species identifications were based on the morphological identification keys (Lane, 1953; Correa and Ramalho, 1956; Harbach, 1994; Forattini, 2002; Sallum et al., 2009). As a result, a total of 3,299 mosquitoes belonging to 41 species were collected. *Plasmodium* vector *Anopheles cruzii* and non-vectors *Ae. serratus*, *Li. durhami*, *Ru. reversa*, and *Wy. quasilongirostris* were found in restinga and forest (Tabela

4.3), in where females may compete for blood sources, such as agoutis or howler monkeys and squirrels, respectively. It was assumed that mosquito blood-feeding relied primarily on host availability (Chaves et al., 2010).

Variations along the altitudinal and vegetational succession gradients were assumed to provide specific resting places after blood-feeding to the egg maturation, which could influence both *An. cruzii* and non-vector species abundances. Altitude and vegetation biomass data were obtained in locations throughout sampled (Bernardi et al., 2010) and thus generalized linear Gaussian geostatistical model with Bayesian inference was run to interpolate the altitude and vegetation biomass in the statistical environment R 2.13 with geoR package (Diggle and Ribeiro, 2006). As a result, in Figura 4.8A light green represented scrubs on plains and hilltops, whereas dark green meant tropical pluvial forest. In Figura 4.8B, lowlands were represented as light brown (< 50 m) and hillsides as dark brown (from 50 to 350 m), whereas hilltops were underrepresented.

Abundance of *An. cruzii* and non-vectors species was, firstly, regressed against values of altitude and vegetation biomass interpolations and, secondly, extrapolated to Parque Estadual da Ilha do Cardoso (Figura 4.9A, Figura 4.9B, Figura 4.9C, Figura 4.9D, Figura 4.9E), based on the the best fitted and parsimonious regression model (Pinheiro, 2011, Wood, 2011) (Tabela 4.4). Zonal statistic in the Spatial Analyst extension of ArcMap (esri.com) was utilized to estimate abundances of *An. cruzii* (X_m^*) as 300 and non-vectors (C) as 3,640 in Marujá fishing village and 1,514 (*An. cruzii*, X_m^*) and 14,101 (non-vectors, C) in indigenous Guarani Mbya settlement.

4.4.4 *Anopheles cruzii* biting rate (b) and mortality rate (μ)

Roseli La Corte dos Santos and its advisor Dr. Forattini estimated the vectorial capacity of mosquitoes of subgenera *Kerteszia* of *Anopheles* in Atlantic Forest, southeastern Brazil (Santos, 2001). The gonotrophic cycle duration was estimated as approximately 4 days under laboratory conditions (Santos, 2001). Considering gonotrophic discordance (Santos, 2001), it was supposed that females could bite two times in average per cycle duration (4 days) which thus led to an estimate of biting rate as 0.5 (i.e., $\frac{\text{number of bites per cycle}}{\text{gonotrophic cycle duration in days}} = \frac{2}{4} = 0.5$ bite/female/day). The daily survival rate was estimated as 0.45 employing mark-release-recapture and physiological age techniques (Santos, 2001). It was assumed that *An. cruzii* population could increase or decrease exponentially in time and thus its daily mortality rate (μ) was equal to minus natural logarithm of daily survival rate (i.e., $\mu = -\log(0.45) = 0.8$).

4.4.5 *Anopheles cruzii* conversion rate (α)

Anopheles cruzii was present which meant that emergence of *An. cruzii* adults in average must surpass its mortality rate (i.e., $\alpha b > \mu$) and abundance of non-vector mosquito species must be smaller than abundance of *An. cruzii* (i.e., $C < \left(\frac{\alpha b}{\mu} - 1\right) h(B + N)$). Equilibrium between abundance of *An. cruzii* and non-vector mosquito species was represented as follow:

$$X_m^* = \left(\frac{\alpha b}{\mu} - 1\right) h(B + N) - C \quad (4.12)$$

where input parameters $1,514 = \left(\frac{\alpha 0.5}{0.8} - 1\right) 20(172 + 150) - 14,101$ led to an estimate of α as 5.5 in indigenous Guarani Mbya settlement and $300 = \left(\frac{\alpha 0.5}{0.8} - 1\right) 20(47 + 165) - 3,640$ led to an estimate of α as 3.1 in Marujá fishing village.

4.4.6 Host tolerance (h)

Success to feed upon a host was assumed to be determined by the lack of host defensiveness responses to biting mosquitoes, which is supported in the work by Kelly (2001). In the present work, host tolerance (h) was estimated as the number of bites without a host defensive behavior per day (10; Figura 4.10) divided by *An. cruzii* biting rate (0.5) (i.e., $h = \frac{10}{0.5} = 20$). To study sensitivity of bromeliad malaria model to host tolerance (h), it was found that if $h \leq 18$ then *An. cruzii* was excluded by competition in both human settlements; if $h \geq 30$ and $h \geq 35$ then $R_0 > 1$ in Marujá fishing village and indigenous Guarani Mbya settlement, respectively. Considering that competitive exclusion of *An. cruzii* and bromeliad malaria invasion ($R_0 > 1$) represented less possible situations, mosquito vector diffuse competition and dead-end parasite transmission patterns were assessed for h values within 20 and 30 (e.g., 21, 25, and 29). As a result, no qualitative changes in the first interpretations (see the main text) could be made (Figura 4.11A-D, Figura 4.12A-D, and Figura 4.13A-D).

Figura 4.5: Human population and its geographical location. Clear-cut areas in the northern part of indigenous Guarani Mbya settlement (G) represent logged forest that are utilized to agriculture. In slopes of the southern part of indigenous Guarani Mbya settlement (G) vertebrate animals can be hunted. Fishermans build houses for their families in Marujá fishing village (M) which are as well utilized as hostels for ecotourists. (Source: Instituto Florestal do Estado de São Paulo, 1998).

Figura 4.6: Occurrence of mammals in Parque Estadual da Ilha do Cardoso. Mammal species were either seen or heard. Footprints were also utilized to indicate their presence. Legend: ●, *Alouatta guariba* (howler monkey); ○, *Mazama americana* (deer); ⊙, *Nasua nasua* (coati); ■, *Pecari tajacu* (collared pecari); □, *Leopardus pardalis*, *L. wiedii* e *Herpailurus yaguarondi* (small spotted cats); ◻, *Sciurus ingrami* (squirrel); ▲, *Cerdocyon thous* (fox); △, *Eira barbara* (tayra); ▴, *Tayassu pecari* (white-lipped pecary); +, *Dasyprocta leporina* (agouti). (Source: Bernardo, 2004.)

Figura 4.7: Occurrence of birds in Parque Estadual da Ilha do Cardoso. Bird species were either seen or heard. Legend: ●, *Ramphastos dicolorus* and *R. vitellinus* (toucans); ○, *Penelope obscura* and *P. superciliaris* (guans); ■, *Pipile jacutinga* (guan); □, *Crypturellus obsoletus* (tinamou); ▲, *Odontophorus capueira* (spot-winged wood quail); △, *Tinamus solitarius* (tinamou). Source: (Bernardo, 2004).

Figura 4.8: Abundance of non-vector mosquito species (*C*) and *Anopheles cruzii* (X_m^*): interpolations of ecologic niche axes. A: Vegetation biomass (m³ of wood per m²); B: Altitude (meters above the sea). Points represent field sampling locations that were utilized for performing interpolations (grid of 200 m-spatial resolution). (Source: Bernardi et al., 2005.)

Tabela 4.2: Animal and bird species, density and population size estimates in the Parque Estadual da Ilha do Cardoso.

Species (popular name)	Animal density (n° of individuals per Km²)	Number of individuals (n°)
<i>Odontophorus capueira</i> (spot-winged wood quail)	26.7 (18.0 - 37.2)	2,963 (2,004 - 4,129)
<i>Ramphastos dicolorus</i> and <i>R. vitellinus</i> (toucans)	12.2 (10.6 - 15.9)	1,354 (1,179 - 1,765)
<i>Alouatta guariba</i> (howler monkey)	8.9 (6.9 - 11.6)	997 (769 - 1,291)
<i>Dasyprocta leporina</i> (agouti)	3.4 (2.3 - 4.4)	385 (264 - 488)
<i>Penelope obscura</i> and <i>P. supercilialis</i> (guans)	3.2 (2.8 - 4.2)	359 (311 - 467)
<i>Pipile jacutinga</i> (guan)	2.1 (1.8 - 2.7)	234 (203 - 304)
<i>Sciurus ingrami</i> (squirrel)	1.6 (1.1 - 2.0)	182 (124 - 231)
<i>Tinamus solitarius</i> (tinamou)	1.2 (1.0 - 1.6)	140 (121 - 182)

Estimates of animal population size and density were performed in the software DISTANCE 4.1. The values in parentheses correspond to the lower and upper limits of 95% confidence interval. (Source: Bernardo, 2004.)

Figura 4.9: Abundance of non-vector mosquito species (*C*) and *Anopheles cruzii* (X_m^*): spatial abundance distribution modelling. A: Abundance of *An. cruzii* (altitude β_1 of 6.65 and vegetation biomass β_2 of 2.13; R^2 -adjusted = 0.91); B: Abundance of *Ae. serratus* (vegetation biomass β_1 of 2.13; R^2 -adjusted = 0.16); C: Abundance of *Li. durhami* (altitude β_1 of 2.88 and vegetation biomass β_2 of 1.00; R^2 -adjusted = 0.93); D: Abundance of *Ru. reversa* (altitude β_1 of 6.4; R^2 -adjusted = 0.25); and E: Abundance of *Wy. quasilongirostris* (altitude β_1 of 5.5; R^2 -adjusted = 0.34; grid of 200 m-spatial resolution). G, indigenous Guarani Mbya settlement; and M, Marujá fishing village.

Figura 4.10: Relationships between successes and attempts in mosquito biting events in a given day. A: Host defensive behavior can avoid biting success. B: Biting attempt is linearly correlated with biting success upon a host.

Figure 4.11: Sensitivity analysis: if $h = 21$ then $R_0 < 1$. A, B: Decrease in abundance of non-vector mosquito species can increase risk of bromeliad malaria transmission ($R_0 > 1$) in indigenous Guarani Mbya settlement and Marujá fishing village, respectively; C, D: Decrease in abundance of non-host vertebrate species does not increase risk of bromeliad malaria transmission ($R_0 < 1$) in indigenous Guarani Mbya settlement and Marujá fishing village, respectively.

Figura 4.12: Sensitivity analysis: if $h = 25$ then $R_0 < 1$. A, B: Decrease in abundance of non-vector mosquito species can increase risk of bromeliad malaria transmission ($R_0 > 1$) in indigenous Guarani Mbya settlement and Marujá fishing village, respectively; C, D: Decrease in abundance of non-host vertebrate species does not increase risk of bromeliad malaria transmission ($R_0 < 1$) in indigenous Guarani Mbya settlement and Marujá fishing village, respectively.

Figura 4.13: Sensitivity analysis: if $h = 29$ then $R_0 < 1$. A, B: Decrease in abundance of non-vector mosquito species can increase risk of bromeliad malaria transmission ($R_0 > 1$) in indigenous Guarani Mbya settlement and Marujá fishing village, respectively; C, D: Decrease in abundance of non-host vertebrate species does not increase risk of bromeliad malaria transmission ($R_0 < 1$) in indigenous Guarani Mbya settlement and Marujá fishing village, respectively. D: Increase in abundance of non-host vertebrate species can increase risk of bromeliad malaria transmission ($R_0 > 1$) in Marujá fishing village, which is supported in the work by Saul (2003).

Tabela 4.3: Number of individuals, mosquito species, and vegetation types in the Parque Estadual da Ilha do Cardoso, southeastern Atlantic Forest of Brazil.

Species	Scrub vegetation	Restinga	Tropical pluvial forest
<i>Aedes albifasciatus</i>	13	0	0
<i>Aedes hastatus</i>	3	18	2
<i>Aedes fulvus</i>	0	2	0
<i>Aedes oligopistus</i>	4	26	2
<i>Aedes scapularis</i>	542	79	6
<i>Aedes serratus</i>	26	160	21
<i>Anopheles bellator</i>	26	9	0
<i>Anopheles cruzii</i>	23	33	90
<i>Anopheles homunculus</i>	1	11	15
<i>Anopheles maculipes</i>	1	0	0
<i>Anopheles mediopunctatus</i>	1	0	1
<i>Coquilleltidia chrysonotum</i>	766	337	93
<i>Coquilleltidia venezuelensis</i>	3	3	1
<i>Culex imitator</i>	1	0	0
<i>Culex inadimirabilis</i>	77	0	0
<i>Culex neglectus</i>	0	2	0
<i>Culex ocossa</i>	2	0	0
<i>Culex ribeirensis</i>	83	0	0
<i>Culex sacchettae</i>	9	15	0
<i>Psorophora albigena</i>	0	9	0
<i>Psorophora albipes</i>	0	8	0
<i>Psorophora ferox</i>	7	64	0
<i>Limatus durhami</i>	0	42	14
<i>Limatus flavisetosus</i>	0	16	3
<i>Runchomyia cerqueirai</i>	0	5	0
<i>Runchomyia frontosa</i>	0	0	3
<i>Runchomyia humboldti</i>	0	4	1
<i>Runchomyia reversa</i>	0	54	1
<i>Runchomyia theobaldi</i>	0	29	2
<i>Sabethes intermedius</i>	0	1	0
<i>Sabethes soperi</i>	0	1	0
<i>Trichoprosopon pallidiventer</i>	0	2	2
<i>Wyeomyia aporonoma</i>	0	4	3
<i>Wyeomyia confusa</i>	0	11	4
<i>Wyeomyia mulhensi</i>	10	137	8
<i>Wyeomyia occulta</i>	0	1	0
<i>Wyeomyia pallidoventer</i>	0	61	0
<i>Wyeomyia pertinens</i>	0	5	1
<i>Wyeomyia quaslongirostris</i>	12	228	18
<i>Wyeomyia shannoni</i>	0	1	0
<i>Wyeomyia theobaldi</i>	0	17	3

Tabela 4.4: Mosquito abundance regression model, independent variables, and Akaike Information Criteria values.

Model	Independent variables	<i>Anopheles cruzii</i>	<i>Aedes serratus</i>	<i>Runchomyia reversa</i>	<i>Limatus durhami</i>	<i>Wyeomyia quaslongirostris</i>
1. GL	Alt	190.89	212.76	165.98	161.03	241.25
2. GL	Bioveg	210.70	209.61	165.72	162.10	239.60
3. GL	Alt + Bioveg	190.83	209.12	162.94	162.19	234.18
4. GL	Alt $\times$ Bioveg	192.15	209.10	164.52	156.58	236.10
5. a-LS	Alt	194.81	211.67	173.20	151.96	232.35
6. a-LS	Bioveg	201.69	209.52	169.21	146.64	228.22
7. a-LS	Alt + Bioveg	195.63	210.22	175.46	154.15	229.01
8. a-LS	Alt $\times$ Bioveg	205.92	219.95	186.86	165.53	238.09
9. NB	Alt	140.35	183.89	111.82	91.83	195.57
10. NB	Bioveg	149.95	178.85	110.87	83.25	189.81
11. NB	Alt + Bioveg	142.10	177.81	100.49	83.87	181.62
12. NB	Alt $\times$ Bioveg	143.95	177.81	86.40	72.64	183.59
13. GA	Alt	139.81	177.94	83.81 ^a	62.92	160.22 ^a
14. GA	Bioveg	149.86	175.37 ^a	86.55	58.41	174.62
15. GA	Alt + Bioveg	136.45 ^a	178.23	84.34	57.34 ^a	164.58
16. GA	Alt $\times$ Bioveg	NC	177.74	NC	NC	NC

Model: GL, Gaussian linear regression model (the simplest model); a-LS, autorregressive least square model (it considered spatial correlation structure); NB, negative binomial regression model (it considered exponential non-linear relationship between mosquito abundance and independent variables); and GA, generalized additive regression model (it considered exponential non-linear relationship and a very flexible smooth function that maximize model fit).

Independent variables: Alt, altitude interpolation; Bioveg, vegetation biomass interpolation; Alt + Bioveg, sum of effects; Alt $\times$ Bioveg, interaction.

NC: model did not converge to a result.

^a: The best model, i.e., the lowest Akaike Information Criteria value.

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DISCUSSÃO E CONCLUSÕES

Os temas e métodos apresentados nos capítulos dessa tese são abordagens de ecologia epidemiológica na Mata Atlântica. Ecologia epidemiológica é o estudo das interações entre vetores, hospedeiros e parasitos na dinâmica de doenças infecciosas.

No capítulo 1, a distribuição potencial de habitats de *Ae. serratus*, *Ae. scapularis* e *Ps. ferox* foi caracterizada no Vale do Ribeira. Os habitats dessas espécies ocorrem em localidades com baixa declividade, apresentando condições climáticas de temperatura máxima elevada e alta precipitação anual. *Aedes serratus* e *Ps. ferox* são dependentes da presença de cobertura vegetal, enquanto que *Ae. scapularis* ocorre em paisagens com menor proporção de mata. As populações humanas residentes na costa Atlântica do Vale do Ribeira estão mais expostas às picadas dessas espécies. A contribuição do presente capítulo para a ecologia e importância epidemiológica dessas espécies (Forattini and Massad, 1998) foi a extrapolação quantitativa da distribuição espacial das mesmas.

Os modelos de distribuição potencial de habitats são ferramentas para o monitoramento de vetores dos arbovírus que circulam no Brasil (Laporta et al., 2012). Para melhor utilizar essa ferramenta na vigilância de arbovirose, recomendam-se: 1, identificar a presença de arbovírus nos mosquitos; 2, construir camadas climáticas atuais com dados de temperatura e precipitação local; 3, aplicar modelo de distribuição de habitats com dados de infecção natural em mosquitos e camadas de vegetação, topografia e clima.

No capítulo seguinte, a distribuição potencial de habitats de *An. bellator*, *An. cruzii* e *An. marajoara* foi caracterizada no Vale do Ribeira. *Anopheles bellator* ocorre na costa Atlântica dessa região, possivelmente associado aos ecossistemas de restinga em que bromélias terrestres expostas ao sol são habitats larvais. *Anopheles cruzii* está correlacionado com locais de alto declive e, assim, a espécie ocorre, principalmente, na Serra do Mar. *Anopheles marajoara* ocorre em áreas sem cobertura vegetal no Vale do Ribeira.

Utilizando modelagem por regressão Poisson, a presença de habitats potenciais para ambos *An. cruzii* e *An. marajoara* está associada com a distribuição de casos autóctones de malária. Portanto, é provável que existam dois ciclos de transmissão de *Plasmodium*, sendo o silvestre com a presença de *An. cruzii* (Curado et al., 2006; Duarte et al., 2008) e o rural com a presença de *An. marajoara* em áreas abertas, naturais (Correa et al., 1950) ou induzidas pelo homem (Laporta et al., 2011), no Vale do Ribeira. Considerando os determinantes de cada ciclo de transmissão, silvestre e rural, o resultado da restauração dos remanescentes de vegetação poderia ser um maior número de casos humanos, acidentais, relacionados ao primeiro, enquanto que o desmatamento poderia contribuir com o surgimento de novos habitats para as espécies de *An. albitarsis* l.s. (Forattini et al., 1995), incluindo *An. marajoara*, provocando, possivelmente, situação de risco de epidemias. Dessa forma, seria importante, em pesquisa futura, determinar a importância do *An. marajoara* e do *An. cruzii* na dinâmica de transmissão de plasmódios humanos no Vale do Ribeira.

No capítulo 3, utilizando os grupos funcionais como mosquitos de solo, mosquito de fitotelmatas (exceto bromélias) e mosquitos de bromélias, verificou-se que a riqueza dos mosquitos de solo esteve correlacionada com a maior heterogeneidade da distribuição local de serrapilheira, o que está em acordo com a teoria de nicho e partição diferencial de recursos (Tokeshi, 1999), possibilitando uma maior coexistência de espécies. A equitabilidade dos mosquitos de solo esteve correla-

cionada com a discrepância entre a quantidade de serrapilheira e a estrutura de vegetação, o que tem suporte com o trabalho de Hubbell (2001) em que maior variação regional pode ter efeito diminuindo a dominância local. Espécies vetoras de parasitos humanos associadas a habitats larvais em zonas rurais, por exemplo, *Anopheles marajoara*, *Mansonia titillans*, *Coquillettidia venezuelensis* e *Culex nigripalpus* (Forattini e Massad, 1998), não foram encontradas. *Aedes scapularis*, que é espécie vetora de arbovírus Rocio (Mitchell et al., 1986), foi coletada em baixa abundância, sendo dominante em 1 de 30 pontos de coleta. A riqueza de mosquitos fitotelmatas está correlacionada com fisionomias vegetais mais estruturadas, p. ex., floresta ombrófila densa. Nesse ambiente, maior heterogeneidade de tipos de habitat larval em fitotelmatas como axilas foliares, bambus, ocos de árvores e epífitas estão mais disponíveis (Forattini, 1965). Ambientes mais homogêneos em fitotelmatas, p. ex., a restinga que tem, principalmente, bromélias terrestres, estão correlacionados com menor riqueza de mosquitos fitotelmatas. As espécies dominantes na restinga (*Runchomyia reversa*) e na floresta (*Limatus durhami*) não são consideradas vetoras de parasitos aos humanos e animais. A abundância total de mosquito de bromélias esteve correlacionada com o número de bromélias, sendo que locais de maior abundância total são os mais ricos em espécies, o que é esperado segundo Yee e Juliano (2007). *Culex imitator* e *Wyeomyia* (*Phonomyia*) sp. foram dominantes na restinga, enquanto que *An. cruzii* foi dominante na floresta. *Anopheles cruzii*, que é considerado vetor de plasmódios no Vale do Ribeira (Coutinho et al., 1947; Curado et al., 2006), foi dominante em pontos na restinga arbustiva, restinga arbórea e floresta ombrófila densa, contendo distribuição homogênea (menor variância) das bromélias terrestres.

Em termos gerais, o capítulo 3 forneceu evidência de que a fauna de mosquitos pode ser utilizada para o monitoramento e conservação da Mata Atlântica. A riqueza total (=soma da riqueza dos três grupos funcionais de mosquitos) é

mais elevada em locais da restinga arbórea (Figura). Esse ambiente representa a transição entre a restinga arbustiva e a floresta, possuindo maior heterogeneidade espacial, ou seja, presença de habitats larvais de solo e de fitotelmatas. Portanto, a heterogeneidade espacial de habitats larvais aumenta a riqueza de espécies de mosquitos. A dominância total (=maior variância das abundâncias relativas da comunidade de mosquitos) é menor na floresta ombrófila densa (Figura). A conservação desse ambiente representa menor risco de emergência de vetores.

Figura: Riqueza total e dominância da comunidade de mosquitos da Ilha do Cardoso segundo a estrutura da vegetação da restinga arbustiva, restinga arbórea e floresta ombrófila densa.

O papel da biodiversidade na dinâmica de transmissão de parasitos da malária na Mata Atlântica foi estudado no quarto capítulo. Como resultado, a transmissão de plasmódios é dificultada pela abundância de mosquitos não-vetores os quais competem com o *An. cruzii* por hospedeiros humanos, o que é competição difusa segundo Pianka (1974). A abundância de aves e mamíferos tem efeito complexo na dinâmica de transmissão, o que tem suporte com o trabalho de Saul (2003), podendo contribuir com a extinção do *Plasmodium* ou aumentando a abundância relativa de *An. cruzii*.

Foi elaborado modelo matemático da dinâmica de transmissão de malária associada a *An. cruzii*. Foi o primeiro modelo de dinâmica de transmissão de malária a considerar a interação ecológica do vetor com a comunidade de mosquito e do hospedeiro-parasito com a comunidade de não-hospedeiros. Para melhorar esse modelo, seriam necessárias as seguintes mudanças: 1, acrescentar outros vetores; 2, acrescentar outros hospedeiros (e.g., reservatório primata); 3, acrescentar as três espécies de plasmódios (i.e., *P. vivax*, *P. falciparum*, *P. malariae*).

Sobretudo, a hipótese mais plausível é a de que a biodiversidade da Mata Atlântica interfere, positiva ou negativamente, na dinâmica de transmissão de malária e arbovirose. Dessa maneira, os programas de conservação e restauração da Mata Atlântica deveriam considerar que seus efeitos poderão causar a emergência dessas doenças infecciosas, por exemplo, facilitando a dispersão de parasitos e seus hospedeiros aves/mamíferos na paisagem, mantendo a circulação de arbovírus e plasmódios em ciclos enzoóticos com a participação de vetores silvestres, infectando acidentalmente os humanos. Paralelamente, os programas de vigilância em saúde e de erradicação da malária deveriam considerar os efeitos da biodiversidade na regulação dessas doenças infecciosas, por exemplo, bloqueando a transmissão dos parasitos por meio de diluição do contingente de hospedeiros na comunidade de vertebrados (efeito de diluição) e de competição por picadas nos hospedeiros entre vetores e não-vetores (efeito da competição difusa).

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ANEXO

A seguir é mostrado artigo desenvolvido para essa tese que foi realizado para estimar a riqueza e a abundância de mosquitos diurnos em remanescentes florestais da Mata Atlântica. Mosquitos diurnos dos gêneros *Haemagogus* e *Sabethes* possuem picos diurnos de atividade hematofágica dentro da mata e, assim, podem exercer papel de vetor primário na dinâmica de transmissão da febre amarela, a qual pode ser mantida em focos naturais em que primatas são reservatórios do vírus. Com a utilização de armadilha CDC e atrativo químico CO₂ liberado em baixa vazão foi possível coletar mosquitos que poderiam picar o homem dentro da mata. As espécies coletadas mais abundantes foram *Aedes serratus*, *Limatus flavisetosus*, *Limatus durhami* e *Wyeomyia confusa*. Dessas espécies, *Ae. serratus* pode ser vetor secundário do vírus da febre amarela quando o homem, acidentalmente, invade o foco natural dessa doença. O trabalho, portanto, recomenda atenção a elevada abundância de *Ae. serratus* e seu possível efeito positivo na transmissão de vírus da febre amarela em áreas rurais da Mata Atlântica.

Effect of CO₂ and 1-octen-3-ol attractants for estimating species richness and the abundance of diurnal mosquitoes in the southeastern Atlantic forest, Brazil

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*Studies have shown that both carbon dioxide (CO₂) and octenol (1-octen-3-ol) are effective attractants for mosquitoes. The objective of the present study was to evaluate the attractiveness of 1-octen-3-ol and CO₂ for diurnal mosquitoes in the southeastern Atlantic forest. A Latin square experimental design was employed with four treatments: CDC-light trap (CDC-LT), CDC-LT and 1-octen-3-ol, CDC-LT and CO₂ and CDC-LT with 1-octen-3-ol and CO₂. Results demonstrated that both CDC-CO₂ and CDC-CO₂-1-octen-3-ol captured a greater number of mosquito species and specimens compared to CDC-1-octen-3-ol; CDC-LT was used as the control. Interestingly, *Anopheles* (Kerteszia) sp. was generally attracted to 1-octen-3-ol, whereas *Aedes serratus* was the most abundant species in all Latin square collections. This species was recently shown to be competent to transmit the yellow fever virus and may therefore play a role as a disease vector in rural areas of Brazil.*

Key words: insect attractants - disease vectors - mosquito control

Carbon dioxide (CO₂) is an effective attractant for collecting mosquitoes (Diptera, Culicidae) and thus for monitoring vector-borne diseases (Reeves 1951, Silver 2008). Octenol (1-octen-3-ol) was first demonstrated to be a mosquito attractant by Takken and Kline (1989). These authors successfully utilised both 1-octen-3-ol and CO₂ to attract *Aedes taeniorhynchus* (Wiedemann) in Everglades National Park, Florida, USA. Later Kline et al. (1990) compared mosquito attraction to 1-octen-3-ol and CO₂ in the Everglades and verified that few mosquito species responded in large numbers to 1-octen-3-ol alone. However, there was an increase in the amount of mosquito species due to the synergistic effect between CO₂ and 1-octen-3-ol. Interestingly, *Culex* sp. showed poor response to 1-octen-3-ol alone or in combination with carbon dioxide. Taken together, Kline et al. (1990) demonstrated that the synergistic effect of both chemicals attracted zoophilic species, whereas the attraction for ornithophilic species, such as *Culex* sp. was poor.

In South America, CO₂ was successfully used for collecting mosquitoes in the Atlantic forest (Forattini et al. 1993a). Nocturnal collections were performed with CDC-CO₂ traps in an irrigated rice field in the Vale do Ribeira, state of São Paulo (SP), Brazil. *Aedes scapularis* (Ron-dani), *Anopheles albitarsis* Lynch Arribálzaga, *Mansonia indubitans* Dyar and Shannon and *Coquillettidia venezuelensis* (Theobald) were abundant. These results may be

explained by the adaptation of mosquitoes to the modified environment and the high concentration of CO₂ used. Although Forattini et al. (1993a) successfully utilized CO₂, this attractant was not yet applied in other circumstances, i.e., diurnal catches with a lower flow rate on a forested site. Reisen et al. (2000) reported that CO₂ is released at an average rate of 500 mL/min for a 1.5-kg block of dry ice in a similar trap. According to Reeves (1953), a high flow rate of CO₂ (> 500 mL/min) attracts mosquito species that usually feed on large mammals. Therefore, a CO₂ flow rate of 250 mL/min is expected to predominately attract anthropophilic species.

Yellow fever virus (YFV) can be transmitted by mosquitoes of the genera *Haemagogus* and *Sabethes* (Vasconcelos 2003), which are common in the Atlantic forest (Forattini 1965). As YFV circulates in SP (de Souza et al. 2010) and may be carried by other vectors (Cardoso et al. 2010), it is important to establish which mosquito species are involved in the dynamics of transmission. Consequently, vector surveillance should employ efficient collection methods that allow for definitive species identification while also providing an accurate representation of species abundance and richness.

Therefore, the objectives of this study were the following: (i) to evaluate a CO₂ collection method in remote areas, (ii) to compare the attractiveness of 1-octen-3-ol alone, CO₂ alone, or CO₂ plus 1-octen-3-ol with a standard CDC-light trap (CDC-LT) for diurnal mosquitoes and (iii) to discuss the possible implementation of these methods for the surveillance of YFV vectors in the southeastern Atlantic forest.

MATERIALS AND METHODS

Study design - A field trial was carried out in a remnant of the Atlantic forest; the selected site was a dense ombrophilous forest in a mountainous landscape (Fig-

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ure). Three collection sites, located 200 m apart, were chosen in Sítio Itapuã, Cananéia, SP, Brazil. Latin square analyses, consisting of one CDC-LT, one CDC-LT with 1-octen-3-ol, one CDC-LT with CO₂ and one CDC-LT with CO₂ plus 1-octen-3-ol were performed at each site.

Mosquito collections were carried out over a 12 h period, from 06:00 am-06:00 pm. Collections were performed in site 1, in the days 8, 10, 17 and 19, in site 2, in the days 24, 26 November, 1, 3 December, and in site 3, in the days 8, 10, 15 and 17 December 2009. Each treatment was tested in all possible positions at each collection site. Previous studies have determined that *Aedes* sp. were attracted at distances of 3 and 7 m from the CO₂ source (McIver & McElligott 1989) and that CDC-LTs placed 15 m apart act as independent traps (Brown et al. 2008). Therefore, to avoid interference among traps, they were placed approximately 16 m apart.

Species identifications were based on the morphological identification keys proposed by Lane (1953), Correa and Ramalho (1956) and Forattini (2002).

Traps - The CDC-LTs employed in this study were identical to those used by Forattini et al. (1993a) with one exception; the four large-sized batteries were replaced by a rechargeable battery, which supplied electric power for the 3 W incandescent bulb and the 6 V engine. The 1-octen-3-ol mosquito attractant (Koolatron™), an alcohol, consisted of a solid strip containing 3.72 g of 1-octen-3-

ol. CO₂ was supplied from a 4.5 kg cylinder (White Martins™). The scheme proposed by Addison et al. (1979) was employed with the following modifications. First, a low-flow valve (Swagelok™) was connected to the cylinder allowing CO₂ release to be adjusted to simulate a human host (250 mL CO₂ per minute) (Reeves 1953). Also, a tube (Swagelok™) 6.25 cm in diameter and 100 cm in length was attached to this valve. This release tube was then connected to the head of the CDC-LT, which was suspended at a height of 1.5 m. All other CDC-light traps were also suspended at a height of 1.5 m.

Species richness - Species richness in each trap was divided by total species richness (Table I) to estimate a third variable, which was employed as the sensibility of the collection method. To compare sensibility among treatments, a multi-model framework was established. Models were as follows: model 1a (neutral model), all treatments were considered to have identical sensibility; model 2a (multiple effects), each treatment has a different sensibility; model 3a (control effect), treatment 1 (control) has a different sensibility, but others have equal sensibility; model 4a (CO₂ effect), treatments 1 and 2 have equal sensibility, which differs from those of treatments 3 and 4; model 5a (1-octen-3-ol effect), treatments 1 and 3 have identical sensibility, which differs from those of treatments 2 and 4; model 6a (synergistic effect) treatment 4 has a different sensibility, but others have equal sensibility.

Abundance - Total abundance was the sum of number of specimens collected using a specific treatment. This variable was considered to have a log-normal distribution. Total abundance was compared among treatments using CDC-LT as a control, and a second multi-model framework was applied. Models were as follows: model 1b (neutral model), all treatments were considered to have identical abundance; model 2b (multiple effects), each treatment has a different abundance; model 3b (control effect), treatment 1 (control) has a different abundance, but others have equal abundance; model 4b (CO₂ effect), treatments 1 and 2 have equal abundance, which differs from those of treatments 3 and 4; model 5b (1-octen-3-ol effect), treatments 1 and 3 have identical abundance, which differs from those of treatments 2 and 4; model 6b (synergistic effect) treatment 4 has a different abundance, but others have equal abundance.

Exploratory analysis - Shannon-Wiener and Simpson-Yule diversity scores were calculated for each treatment at each collection site. Analysis of variance was performed in order to compare each of these alpha diversity scores among sites of collection.

Model selection analysis - All analyses were conducted with the software package R 2.9.2 (<http://www.r-project.org.br/>). The Akaike Information Criteria (AIC) was applied to differentiate distances among models in each multi-model framework. AIC values were relative distances among observed models from the real or expected model, which was based on the available data. Moreover, the AIC value was used as a measure of plau-

Sítio Itapuã, Cananéia, Vale do Ribeira (47°50'20W 24°51'48S, South American Datum 69); reference map: the southeastern Atlantic forest (source: National Institute for Space Research).

sibility for each model. If the difference between two AIC values was more than 2, the model with a lower AIC value was considered to be more plausible than the others. One advantage of applying AIC to evaluate collection methods is the use of many concurrent models that might explain observed differences. Here, six concurrent models, 1a-6a and 1b-6b, were each evaluated for richness and abundance among treatments. In addition, model selection was applied for each collection site. Further information about this approach has been published previously (Hobbs & Hilborn 2006).

RESULTS

Collections generated 48 sample units, representing 576 h of effort. As no male mosquitoes were collected, all results represent female mosquitoes. A total of 3,507 mosquitoes belonging to 33 species were collected (Table I). No differences in individual alpha diversity scores were observed among collection sites (Table II). Additionally, no difference in model selection was observed among sites of collection (Supplementary data). Therefore, model selection analysis was performed with richness and abundance data pooled from all collection sites.

TABLE I
Species, type of treatment (T) and number of females, Atlantic forest, 2009

Species	T1 ^a	T2 ^b	T3 ^c	T4 ^d	Total
<i>Aedes (Howardina) fulvithorax</i>	0	1	0	1	2
<i>Aedes (Ochlerotatus) hastatus</i>	0	0	8	7	15
<i>Aedes (Ochlerotatus) scapularis</i>	0	1	8	9	18
<i>Aedes (Ochlerotatus) serratus</i>	7	16	368	308	699
<i>Aedes (Protomacleana) terrens</i>	0	1	7	2	10
<i>Anopheles (Anopheles) mediopunctatus</i>	0	0	0	1	1
<i>Anopheles (Kerteszia) bellator</i>	0	3	30	60	93
<i>Anopheles (Kerteszia) cruzii</i>	0	0	24	74	98
<i>Anopheles (Kerteszia) homunculus</i>	0	0	8	25	33
<i>Coquillettidia (Rhynchoetania) chrysonotum</i>	1	4	17	42	64
<i>Culex (Culex) bidens</i>	0	0	6	4	10
<i>Culex (Melanoconion) sacchettae</i>	0	0	22	7	29
<i>Limatus durhamii</i>	23	8	200	198	429
<i>Limatus flavisetosus</i>	4	1	459	215	679
<i>Onirion personatum</i>	0	0	1	0	1
<i>Psorophora (Janthinosoma) albigena</i>	0	2	1	7	10
<i>Psorophora (Janthinosoma) ferox</i>	2	15	45	47	109
<i>Runchomyia (Runchomyia) cerqueirai</i>	0	0	1	0	1
<i>Runchomyia (Runchomyia) frontosa</i>	0	0	15	9	24
<i>Runchomyia (Runchomyia) reversa</i>	0	0	19	5	24
<i>Runchomyia (Runchomyia) theobaldi</i>	1	0	8	4	13
<i>Sabethes (Peytonulus) soperi</i>	0	0	5	2	7
<i>Sabethes (Sabethinus) intermedius</i>	0	0	2	0	2
<i>Trichoprosopon pallidiventer</i>	0	1	23	8	32
<i>Trichoprosopon townsendi</i>	0	0	1	0	1
<i>Wyeomyia aporonomia</i>	0	0	159	102	261
<i>Wyeomyia occulta</i>	0	0	31	24	55
<i>Wyeomyia (Phoniomyia) pallidiventer</i>	0	0	24	18	42
<i>Wyeomyia (Phoniomyia) quasilongirostris</i>	0	0	15	4	19
<i>Wyeomyia (Phoniomyia) theobaldi</i>	0	0	0	4	4
<i>Wyeomyia (Prosopolepis) confusa</i>	24	10	279	169	482
<i>Wyeomyia shannoni</i>	0	0	39	11	50
<i>Wyeomyia (Wyeomyia) pertinans</i>	0	0	124	66	190
Total abundance	62	63	1,949	1,433	3,507
Total species richness	7	12	30	29	33

a: CDC-light trap; b: CDC-octenol (1-octen-3-ol); c: CDC-carbon dioxide (CO₂); d: CDC-CO₂-1-octen-3-ol.

TABLE II
Analysis of variance of alpha diversity scores

Treatments	Shannon-Wiener	Simpson-Yule
CDC-LT ^a	1.12	0.62
CDC-1-octen-3-ol ^a	1.68	0.76
CDC-CO ₂ ^a	2.12	0.82
CDC-CO ₂ -1-octen-3-ol ^a	2.16	0.82
CDC-LT ^b	1.08	0.62
CDC-1-octen-3-ol ^b	1.55	0.78
CDC-CO ₂ ^b	2.37	0.87
CDC-CO ₂ -1-octen-3-ol ^b	2.39	0.87
CDC-LT ^c	1.12	0.61
CDC-1-octen-3-ol ^c	1.84	0.83
CDC-CO ₂ ^c	2.33	0.86
CDC-CO ₂ -1-octen-3-ol ^c	2.57	0.90
ANOVA one-way	F = 0.11 (p < 0.89) F = 0.14 (p < 0.87)	

a: treatment applied in collection site 1; b: treatment applied in collection site 2; c: treatment applied in collection site 3; CDC-LT: CDC-light trap; CO₂: carbon dioxide; 1-octen-3-ol: octenol.

Species richness - Collected species predominately belonged to the Sabethini and Aedini tribes. The species richness was seven for CDC-LT, 12 for CDC-1-octen-3-ol, 30 for CDC-CO₂ and 29 for CDC-CO₂-1-octen-3-ol, and sensibility was 21%, 36%, 91% and 88%, respectively. In the first multi-model framework, models 1a (AIC = 72), 2a (AIC = 30), 3a (AIC = 26), 4a (AIC = 32), 5a (AIC = 56) and 6a (AIC = 59) showed great divergence. The most plausible model was 3a (control effect), which was followed by the equally plausible models 2a (multiple effects) and 4a (CO₂ effect). Models 1a (neutral model), 5a (1-octen-3-ol effect) and 6a (synergistic effect) were not plausible.

Abundance - Mosquito abundance varied among treatments (Table I). High relative abundance of *Aedes serratus* (Theobald) (20%), *Limatus flavisetosus* Oliveira Castro (19%), *Wyeomyia confusa* (Lutz) (14.5%) and *Limatus durhami* Theobald (12%) was observed. Numbers of *Ae. serratus*, *Li. flavisetosus*, and *Wy. confusa* were highest in treatment 3 (368, 459 and 482, respectively). The log-natural means and the standard deviations of abundance among the three collection sites were 2.95 ± 0.5 (CDC-LT), 2.75 ± 0.92 (CDC-1-octen-3-ol), 6.43 ± 0.36 (CDC-CO₂) and 6.15 ± 0.17 (CDC-CO₂-1-octen-3-ol). Results of the second multi-model framework were as follows: models 1b (AIC = 673), 2b (AIC = 20), 3b (AIC = 12), 4b (AIC = 44), 5b (AIC = 235) and 6b (AIC = 235). The most plausible model was 3b (control effect), which was followed by models 2b (multiple effects) and 4b (CO₂ effect). Models 1b (neutral model), 5b (1-octen-3-ol effect) and 6b (synergistic effect) were not plausible. Although total abundance was not correlated to 1-octen-3-ol presence, *Anopheles (Kerteszia)* sp. responded well to this attractant. For example, CDC-CO₂-1-octen-3-ol

collected three times more *Anopheles cruzii* Dyar and Knab and two times more *Anopheles bellator* Dyar and Knab compared to CDC-CO₂ (Table I). Further, while 25 specimens of *Anopheles homunculus* Komp were collected using CDC-CO₂-1-octen-3-ol, none were collected in the CDC-LT.

DISCUSSION

First and second multi-model frameworks showed similar results. Neutral models 1a (AIC = 72) and 1b (AIC = 673) were the least plausible. Thus, the results of sensibility and abundance among treatments were not merely caused by random effects. Models 3a (AIC = 26) and 3b (AIC = 12) of the control effect were very plausible and showed that the sensibility and abundance of the CDC-LT were different from other traps. The CDC-LT was an efficient control and the attractants 1-octen-3-ol and CO₂ influenced the capture of mosquitoes. Models 2a (AIC = 30) and 4a (AIC = 32) were equally plausible, indicating that each treatment had a different sensibility and that the contribution of CO₂ was strong. Models 2b (AIC = 20) and 4b (AIC = 44) suggested that abundance was different for each treatment and that there was a high CO₂ effect. The CDC-CO₂ trap had the highest species richness (30), sensibility (91%) and total abundance (1,949). There was not a relevant 1-octen-3-ol effect (models 5a, AIC = 56 and 5b, AIC = 235) or synergistic effect (models 6a, AIC = 59 and 6b, AIC = 235). However, the nocturnal mosquitoes *An. cruzii* and *An. bellator*, which are *Plasmodium* sp. vectors (Marrelli et al. 2007), were caught at higher frequencies in the CDC-CO₂-1-octen-3-ol trap. Although, Rubio-Palis (1996) stated that 1-octen-3-ol is best omitted as an attractant when working with *Anopheles albimanus* Wiedemann and *Anopheles aquasalis* Curry, the overall ineffective contribution of 1-octen-3-ol to the collection of diurnal mosquitoes was not expected.

In our study, the 1-octen-3-ol emission rate was approximately 0.05 mg per hour. This emission rate is equivalent to that of an ox (0.025-0.043 mg/h) (Clements 1999). Kline et al. (1990) successfully collected mosquitoes using 3 mg/h of 1-octen-3-ol to simulate the amount released by 60 oxen. In addition, Becker et al. (1995) employed an even greater amount of 1-octen-3-ol (13 mg/h) in their studies. In this work, despite the high concentrations of 1-octen-3-ol used, CDC-1-octen-3-ol performed poorly when compared to CDC-CO₂. In conclusion, both small amounts (0.05 mg/h) and high quantities (13 mg/h) of 1-octen-3-ol appear to be inadequate to attract mosquitoes.

Carestia and Savage (1967) compared the CDC-LT to the CDC-CO₂ trap and, employing the total amount of mosquito species collected, found the sensibility of the CDC-LT to be 45%. However, in the present study, the sensibility of the CDC-LT (21%) and of CDC-1-octen-3-ol (36%) was less than the 45% obtained by Carestia and Savage (1967). Additionally, the 91% sensibility of CDC-CO₂ and 88% of CDC-CO₂-1-octen-3-ol were higher than the expected 45%. As listed in Table I, CDC-CO₂ and CDC-CO₂-1-octen-3-ol shared 26 species of 33

species captured; *Aedes fulvithorax* (Lutz), *Anopheles mediopunctatus* (Lutz), *Onirion personatum* (Lutz), *Runchomyia cerqueirai* Stone, *Sabethes intermedius* (Lutz), *Trichoprosopon townsendi* Stone and *Wyeomyia theobaldi* (Lane and Cerqueira) were not shared by the CDC-CO₂ and CDC-CO₂-1-octen-3-ol traps. Consequently, it is plausible to hypothesize that the sensibility of CDC-CO₂-1-octen-3-ol had a stronger contribution of CO₂ than the synergistic effect of CO₂ plus 1-octen-3-ol.

The synergistic effect is based on the neural excitement of mosquitoes stimulated by CO₂ and 1-octen-3-ol. Maxillary palps of mosquitoes contain sensory neurons that respond to small changes in CO₂ concentration in the air. Additionally, 1-octen-3-ol sensitive neurons are present on the antennae of females (Clements 1999). Stimulation of both CO₂-palp and 1-octen-3-ol antennae can increase mosquito response to attractants. Low emission rates of 1-octen-3-ol (0.05 mg/h) might avoid the synergism with CO₂. Although Kline et al. (1990) observed a synergistic effect with mosquito species in Florida, Becker et al. (1995) did not find CO₂ synergism in Germany. These authors collected more specimens of *Aedes vexans* (Meigen) and *Aedes rossicus* Dolbetski, Gorickaja and Mitrofanova when using a CDC-CO₂ trap compared to the CDC-CO₂-1-octen-3-ol trap. In addition, the synergistic effect of CO₂ (dry ice 100 g/h) and 1-octen-3-ol (0.092 ± 0.015 mg/h) was significantly different from human attraction for collecting *An. albimanus* and *An. aquasalis* (Rubio-Palis 1996).

The release rate of 1-octen-3-ol must be investigated further before its synergistic effect with CO₂ can be used in traps for monitoring diurnal mosquitoes in the southeastern Atlantic forest. Few *Sabethes* specimens and no *Haemagogus* mosquitoes were collected in the entire study (Table I). Further, in the present study area, Ueno (2000) collected *Wyeomyia* sp., *Aedes crinifer* (Theobald) and *Psorophora albipes* (Theobald) mosquitoes using a Shannon trap; these species were not collected in this study. Nevertheless, CDC-CO₂ was an effective attractant for other diurnal mosquitoes. The flow of CO₂ at 250 mL/min was equivalent to the amount released by a human-sized animal placed nearby the CDC trap (Reeves 1953).

The most abundant species collected in the CDC-CO₂ traps, *Ae. serratus*, feeds on humans inside the forest (Forattini et al. 1989). YFV was isolated from *Ae. serratus* specimens collected in the state of Rio Grande do Sul in 2008 (Cardoso et al. 2010). If YFV is introduced into the Atlantic forest, then *Ae. serratus* could act as a vector. Moreover, this pan-tropical mosquito species (Forattini 2002) adapts well to rural areas (Forattini et al. 1993b) and is already a vector for YFV in human-modified environments.

Some mosquitoes collected by Forattini et al. (1993a) were either not present or collected in low frequencies in the present study (e.g., *Ae. scapularis*). This can be explained by the nocturnal feeding activity of *An. albimanus*, *Ma. indubitans*, and *Cq. venezuelensis* and the association of these species and *Ae. scapularis* with human-modified environments. However, the diurnal sylvatic mosquitoes *Li. durhami*, *Li. flavisetosus* and *Wy. confusa* were frequently collected in the CDC-CO₂ traps.

These species are associated with the abundant phytotelmata in the Atlantic forest (Forattini 1965). These species (*Li. durhami*, *Li. flavisetosus* and *Wy. confusa*) along with *Sabethes* sp., which can act as vectors of YFV (Vasconcelos 2003), share similar bionomics characteristics; for instance, they are both diurnal sylvatic species that are abundant in Atlantic forest. In addition, CDC-CO₂ seems to be a very efficient method for monitoring the abundance of these species (*Li. durhami*, *Li. flavisetosus* and *Wy. confusa*) as well as *Ae. serratus*. Moreover, the method described herein can be used in remote areas where dry ice is usually unavailable.

Other studies have compared CDC-CO₂ with Mosquito Magnet™. In relation to total abundance, Mosquito Magnet outperformed CDC-CO₂ (Johansen et al. 2003, Xue et al. 2008). However, the sensibility of CDC-CO₂ (19/27) was very close to that of Mosquito Magnet (20/27) in the study by Johansen et al. (2003). Moreover, in another study, 13 species were collected by both CDC-CO₂ and Mosquito Magnet (Xue et al. 2008). Therefore, CDC-CO₂ should be compared with Mosquito Magnet in the southeastern Atlantic forest to evaluate the best available method of surveillance of adult females of mosquito species involved in the dynamics of parasite transmission to humans and sylvatic animals.

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Formação acadêmica/Titulação

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Bolsista do(a): Fundação de Amparo à Pesquisa do Estado de São Paulo ,FAPESP ,Brasil .
Palavras-chave: Analise espacial; Biodiversidade; ecologia; Culicidae.
Grande área: Ciências Biológicas / *Área:* Ecologia.
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Título: Determinantes de ampla escala das associações entre insetos e plantas: tefritídeos (Diptera) endófagos de capítulos de asteráceas, *Ano de Obtenção:* 1999.
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Palavras-chave: Interação Inseto-Planta; Biotipos; Morfometria; Tephritidae; Asteraceae.
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