UNIVERSIDADE FEDERAL DE PERNAMBUCO
CENTRO DE BIOCIÊNCIAS
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PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA ANIMAL

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Aspectos da Distribuição Geográfica, Estrutura Populacional e Resposta à Competição de Espécies Nativas e Exóticas de Dípteros (Calliphoridae) de Importância Forense

RECIFE 2017

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Tese apresentada ao programa de Pós-Graduação em Biologia Animal da Universidade Federal de Pernambuco, como um dos requisitos para a obtenção do título de Doutor em Biologia Animal.

Orientador: Prof. Dr. Simão Dias de Vasconcelos

RECIFE, 2017

Catalogação na fonte
Elaine Barroso
CRB 1728

Carmo, Rodrigo Felipe Rodrigues do
Aspectos da distribuição geográfica, estrutura populacional e resposta à competição de espécies nativas e exóticas de dípteros (Calliphoridae) de importância forense / Recife: O Autor, 2017.

118 folhas: il., fig., tab.
Orientador: Simão Dias de Vasconcelos
Tese (doutorado) – Universidade Federal de Pernambuco. Centro de Biociências. Biologia Animal, 2017.
Inclui referências e anexos

1. Entomologia forense 2. Díptero 3. Mosca-varejeira I. Vasconcelos, Simão Dias de (orient.) II. Título

595.7 CDD (22.ed.) UFPE/CCB-2017- 475

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Àqueles que
São o alicerce da minha vida
E dedicam tempo e energia ao meu crescimento
Dedico;
João Laurindo, Kátia Rejane,
Raiana Rodrigues & Mayara Vilela

AGRADECIMENTOS

Agradeço a Deus por estar presente em todos os momentos da minha vida, guiando-a e propiciando momentos inesquecíveis!

Seria hipócrita em dizer que esse trabalho é meu, somente. Muitas pessoas estão envolvidas com a minha formação e várias dessas, diretamente relacionadas com o trabalho final do doutorado.

Em primeiro lugar agradeço ao Dr. Simão Vasconcelos – meu orientador, responsável direto pela minha formação profissional como pesquisador, estimulando sempre o desenvolvimento do pensamento crítico e hipotético-dedutivo necessário para um bom cientista. O Dr. Simão, não somente me estimulou como pesquisador, mas também ensinou princípios básicos de trabalho em equipe, sendo coordenador do grupo de pesquisa. Aos colegas de laboratório e amigos pessoais Diego Oliveira e Taciano Moura, pelos debates, crescimento conjunto e auxílio em etapas cruciais para a feitura do trabalho, estando presentes desde a coleta dos dados, até as triagens e identificações do material biológico. Estendo também esses agradecimentos a Paulo Dias. Ao grande amigo Thiago Soares, juntos desde o mestrado, agradeço.

Atrelado a esse trabalho está um esforço colaborativo realizado por parcerias com excelentes pesquisadores/especialistas em cada área de atuação. Seguindo uma ordem numérica dos capítulos que serão apresentados a você posteriormente, agradeço primeiramente ao Dr. José Roberto Botelho Souza (Betinho), não somente por todas as conversas bastante produtivas ao longo desse período acerca de ecologia e dos mais diversos assuntos, fundamentais para a estruturação e finalização do primeiro capítulo da minha tese, mas também pelo ensinamento da simplicidade e auxílio dentro do meio acadêmico. Ao Dr. Diego Astúa, agradeço pela dedicação empenho, além da maestria no qual desenvolve suas pesquisas na área de morfometria. O Dr. Diego foi essencial para a elaboração do segundo capítulo dessa tese, quando após uma disciplina do curso me mostrou um outro ponto de vista sobre os meus dados. Por fim, mas não menos importante, o terceiro capítulo só foi possível ser desenvolvido graças a parceria firmada com o Dr. Jeffery Tomberlin da Texas A&M University (TAMU). Agradeço ao Dr. Tomberlin orientador por um período de 12 meses nos quais passei em seu laboratório aprendendo mais acerca de Ecologia de Dípteros Necrófagos e Entomologia Forense. O agradeço por me receber de braços abertos em seu laboratório e ser fundamental na melhoria do meu desempenho profissional. Nesse período fui presenteado com grandes amigos, os quais posso ter a honra de incluir o Dr. Tomberlin.

Ainda na TAMU, tive a ajuda honrosa de pesquisadores excelentes no desenvolvimento do trabalho proposto, agradeço de forma muito especial ao Dr. Rodrigo Correa pelas horas passadas no laboratório no processo de medição das larvas, além de todas as conversas filosóficas sobre ciência e vida... Nessas conversas eu acrescento meus agradecimentos ao grande amigo Dr. Heo Chong Chin, grandes debates envolvendo filosofia, ecologia e desenho experimental regavam nossos cafés da tarde. Pelo acolhimento em uma terra desconhecida, além dos ensinamentos valiosos sobre bionomia e ciclo de vida de moscas varejeiras agradeço a Ashleigh Faris e ao Dr. Jonathan Cammack. Além deles, Chelsea Holcomb e Aline Espindola por me auxiliarem na coleta de dados.

De volta ao Brasil, agradeço ao Programa de Pós-Graduação em Biologia Animal, aqui representados pelos seus coordenadores, técnicos e Professores, pelo grande empenho dessa equipe no processo de formação de recursos humanos de excelência. Em especial a coordenadora Dra. Bruna Bezerra, por uma gestão impecável agindo de forma séria e célere nos processos burocráticos do programa, não esquecendo de ser humana; ao Dr. Enrico Bernard por estimular de forma indireta o desenvolvimento do pensamento científico, mostrando pontos de vistas distintos daqueles já habituais.

À Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE), agradeço pelo suporte financeiro, concedendo a bolsa de estudos ao longo de todo o período do doutorado. Bem como à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior Conselho (CAPES) pela concessão da bolsa de Doutorado-Sanduíche no Exterior por um período de 12 meses.

Por fim, mas servindo com alicerce de vida agradeço a minha família! Meu Pai João Laurindo, grande mentor e amigo, agradeço por todos os ensinamentos de vida e por ser agente direto na formação do meu caráter. A minha grande amiga e mãe, Kátia Rejane, agradeço por todas as conversas, ensinamentos e por me mostrar que a vida pode ser mais simples do que a gente pensa. A minha irmã, Raiana Rodrigues agradeço por me oportunizar momentos ímpares de alegria e aperreios! Finalmente, àquela que trouxe uma beleza singular para a minha vida, presente na fase final do trabalho, mas me conhecendo como ninguém e me suportando em tudo, Mayara Vilela minha esposa, agradeço pelo simples fato de você existir em mim.

RESUMO

Insetos necrófagos apresentam um importante papel no processo de decomposição da matéria orgânica animal, acelerando a ciclagem de nutrientes. Associado a isso, esses insetos representam uma importante ferramenta nos processos de investigação criminal, servindo como base para as estimativas do intervalo pós morte. A família Calliphoridae se destaca dentre os insetos necrófagos dada seu pioneirismo na colonização do recurso além da íntima associação com o processo de decomposição. O objetivo maior desse trabalho é descrever os padrões e entender os fatores estruturadores da diversidade na montagem das comunidades de dípteros necrófagos ao longo de um gradiente ecológico no Nordeste brasileiro, analisando também, os processos por trás das relações de co-ocorrência de espécies em um mesmo recurso. Para tanto, coletas sistematizadas foram realizadas ao longo de um gradiente longitudinal em Pernambuco. Além disso, 5 populações de *Chrysomya megacephala* e *Cochliomyia macellaria* foram selecionadas como modelos analíticos para a avaliação populacional através do uso de técnicas de morfometria geométrica alar. Por fim, a co-ocorrência de espécies foi acessada através do modelo *Chrysomya rufifacies* e *C. macellaria* afim de testar os efeitos da competição da espécie invasora nos parâmetros bionômicos e de sobrevivência da espécie nativa. Ao serem combinadas todas as 150 unidades amostrais, um total de 33.834 dípteros adultos distribuídos em 10 espécies da Calliphoridae foram registrados. A estruturação das comunidades se deu de forma distinta, com Mata Atlântica sendo a mais diversa apresentando 8 das 10 espécies amostradas. Por outro lado, Ilha oceânica apresentou um padrão claro de dominância da espécie invasora, refletindo na baixa diversidade local. Uma forte tendência de segregação entre as comunidades é evidenciada pelo ANOSIM (R_{global} = 0,914; $P < 0,001$) o que caracteriza especialidades na montagem das comunidades. De uma forma geral, as asas de *C. megacephala* e *C. macellaria* variaram tanto no tamanho quanto na forma ao longo do gradiente longitudinal. Os fatores ambientais influenciaram diferentemente os padrões de asa para *C. megacephala* e *C. macellaria*. Para a primeira, umidade relativa e temperatura máxima foram os fatores que melhor explicaram a diferença no tamanho e forma das asas, enquanto que para *C. macellaria*, altitude e amplitude térmica foram os fatores que melhor explicaram a variação. Em relação ao modelo de coexistência (competição) na carcaça, a simples presença de *Chrysomya rufifacies* foi suficiente para alterar todos os parâmetros (larval e adultos) da espécie nativa. A sobrevivência de *C. macellaria* foi inversamente proporcional a presença de *C. rufifacies* atingindo o nível zero em elevadas proporções da espécie invasora.

Palavras-chave: Insetos Necrófagos. Ecologia de Comunidades. Bionomia Larval. Morfometria Geométrica. Competição.

ABSTRACT

Necrophagous insects play an important role in the decomposition process of animal organic matter, accelerating the nutrient cycling. Associated with this, such insects represent an important tool in criminal investigation processes, serving as a basis for the estimates of the postmortem interval. The Calliphoridae family stands out among the necrophagous insects given its pioneering nature in the colonization of the resource as well as the intimate association with the decomposition process. The main objective of this work is to describe the patterns and to understand the structural factors of the diversity in the assembly of the communities of necrophagous diptera along an ecological gradient in the Northeast of Brazil, also analyzing the processes behind the co-occurrence relations of species in a same resource. For this purpose, systematized collections were carried out along a longitudinal gradient in Pernambuco. In addition, 5 populations *of Chrysomya megacephala* and *Cochliomyia macellaria* were selected as analytical models for the population evaluation using geometric morphometry techniques. Finally, the co-occurrence of species was accessed through the *Chrysomya rufifacies* and *C. macellaria* model in order to test the effects of the competition of the invasive species on the bionomic parameters and the native species survival. When all 150 sample units were combined, a total of 33,834 adult dipterans distributed in 10 species of Calliphoridae were recorded. The structuring of the communities occurred in a different way, with Atlantic Forest being the most diverse presenting 8 of the 10 species sampled. On the other hand, Oceanic island presented a clear pattern of dominance of the invasive species, reflecting in the low local diversity. A strong tendency of segregation between communities is evidenced by ANOSIM (Rglobal = 0.914; P <0.001), which characterizes specialties in the assembly of communities. In general, the wings of *C. megacephala* and *C. macellaria* varied in both size and shape along the longitudinal gradient. Environmental factors influenced wing patterns differently for *C. megacephala* and *C. macellaria*. For the first, relative humidity and maximum temperature were the factors that best explained the difference in the size and shape of the wings, whereas for *C. macellaria*, altitude and thermal amplitude were the factors that best explained the variation. Regarding the model of coexistence (competition) in the carcass, the simple presence of *Chrysomya rufifacies* was enough to alter all the parameters (larval and adult) of the native species. The survival of *C.*

macellaria was inversely proportional to the presence of *C. rufifacies* reaching level zero in high proportions of the invasive species.

Key-words: Necrophagous Insects. Community Ecology. Larval Bionomic. Geometric Morphometrics. Competition.

SUMÁRIO

CAPÍTULO 3

APRESENTAÇÃO

Estudos envolvendo ecologia de dípteros necrófagos podem seguir duas linhas básicas de investigação. A primeira leva em consideração os aspectos por trás do processo efêmero de decomposição da matéria orgânica, tendo como alvo a carcaça ou cadáver, recurso necessário para a manutenção das vidas desses insetos. A segunda linha de abordagem, alvo do presente trabalho, tem como objetivo principal a análise focada no organismo e sua relação com o microcosmo (carcaça ou cadáver) e por consequência, o ambiente em que ele se encontra. A análise dos padrões de diversidade, abundância e distribuição desses insetos na natureza reflete em um melhor entendimento dos mecanismos que permitem que diferentes espécies coexistam em um mesmo recurso/ambiente. Aliado a isso, as relações entre as espécies que co-ocorrem no microcosmo podem elucidar padrões de colonização, estando eles intimamente atrelados ao processo de decomposição.

O objetivo maior desse trabalho é descrever os padrões e entender os fatores estruturadores da diversidade na montagem das comunidades de dípteros necrófagos ao longo de um gradiente ecológico no Nordeste brasileiro, analisando também, os processos por trás das relações de co-ocorrência de espécies em um mesmo microcosmo. A tese foi então estruturada em três capítulos semi-independentes partindo desde análises ecológicas de comunidades, até às relações interespecíficas desses insetos. Para tanto, utilizamos a família Calliphoridae como modelo em todos os estudos do presente trabalho. Apenas o capítulo 1 está redigido em português seguindo as normas do Programa de Pós-Graduação em Biologia Animal. Os capítulos subsequentes estão escritos em língua inglesa seguindo as normas para publicação dos periódicos *Entomological Science* e *Ecological Entomology*, respectivamente. O capítulo 2 já se encontra aceito para publicação, e o terceiro já foi submetido, estando em processo de revisão.

O primeiro capítulo envolve a análise dos padrões de abundância, riqueza e distribuição de dípteros califorídeos (fatores essenciais para a montagem das comunidades locais) ao longo de um gradiente ecológico longitudinal do Nordeste brasileiro. As análises partiram desde o arquipélago de Fernando de Noronha até o extremo oeste do estado, no município de Petrolina, englobando ambientes costeiros, área urbana, caatinga hipoxerófila, caatinga hiperxerófila, além de agro ecossistemas. A pergunta que norteou as análises foi: como se dá a estruturação das assembleias de Calliphoridae ao longo de diferentes paisagens do Nordeste brasileiro? De uma forma geral, o padrão de abundância e riqueza denotou uma caracterização diferenciada das assembleias de Calliphoridae ao longo dos ambientes testados para o Nordeste brasileiro. Pelo menos uma espécie pertencente ao gênero invasor *Chrysomya* esteve presente entre as espécies dominantes para todos os ambientes. Por outro lado, espécies como *Hemilucilia segmentaria* e *Mesembrinella bicolor* foram exclusivas em determinadas fitofisionomias. Nossos resultados apontam para a utilização cautelosa de espécies de Calliphoridae como indicadoras de local de morte. Nesse capítulo, foi firmada a parceria com o Dr. José Roberto Botelho Souza (UFPE), especialista em ecologia de comunidades, cuja contribuição aprofundou as ferramentas analíticas e interpretativas dos resultados.

O segundo capítulo foi construído através da parceria estabelecida com o Dr. Diego Astúa da UFPE, especialista em morfometria geométrica a partir da disciplina oferecida no Programa de Pós-Graduação em Biologia Animal (UFPE). Esse capítulo objetivou principalmente analisar a influência de variáveis ambientais na morfologia (tamanho e forma das asas) de duas espécies de moscas varejeiras, sendo uma nativa *Cochliomyia macellaria* e uma invasora *Chrysomya megacephala*. Para tanto, cinco populações contendo 60 indivíduos de cada espécie (30 machos e 30 fêmeas) foram utilizados nas análises. O tamanho do centroide e as coordenadas dos marcos anatômicos foram utilizados como medidas analíticas para o tamanho e forma das asas, respectivamente. As espécies responderam de forma diferenciada às variáveis ambientais, sendo as variações do tamanho das asas da espécie nativa melhor explicada pela altitude, enquanto a variação no tamanho das asas de *C. megacephala* esteve mais relacionada com a pluviosidade e a temperatura. Para a forma das asas, altitude e temperatura foram os fatores que melhor explicaram a diferença em *C. macellaria*, enquanto para a espécie invasora a pluviosidade foi o fator mais relacionado com essa variação. Esse capítulo foi submetido e aceito para publicação no periódico *Acta Zoologica*, sendo dessa forma redigido em língua inglesa, e acompanhado das respectivas instruções para autores (Anexo).

Ainda dentro do eixo da ecologia de dípteros necrófagos, o terceiro capítulo focou na relação de co-ocorrência de espécies distintas no recurso alimentar, utilizando como modelo experimental uma espécie nativa *C. macellaria* e uma espécie invasora *C. rufifacies*. Todo o experimento foi desenvolvido em laboratório na Texas A&M University (Texas-EUA) em parceria estabelecida com o Dr. Jeffery Tomberlin, através da concessão da bolsa de Doutorado Sanduíche da CAPES por um período de 12 meses. O objetivo principal do estudo foi avaliar Ba história de vida da espécie nativa frente a uma competidora invasora em situações de delimitação de recurso. Ao todo, seis tratamentos foram realizados com diferentes proporções larvais. A simples presença da espécie invasora no mesmo recurso foi suficiente para afetar negativamente todas as variáveis (tamanho e peso das larvas, tempo de desenvolvimento até pupa, peso seco dos adultos, tamanho e forma das asas) testadas para a espécie nativa. Essa influência foi gradativamente elevada com o aumento das proporções larvais (invasora/nativa), ao ponto de existir mortalidade de 100% dos indivíduos de *C. macellaria* em elevadas proporções de *C. rufifacies*. Esse capítulo, assim como o segundo, foi redigido em língua inglesa seguindo os moldes do periódico *Ecological Entomology*. Revista para qual o capítulo foi submetido, e acompanhado das respectivas instruções para autores (Anexo).

Optou-se pelo formato de capítulos independentes para priorizar a submissão dos manuscritos para publicação, deforma que a leitura não segue necessariamente a ordem sequencial, e todas as referências são citadas ao final de cada capítulo. Material suplementar está também disponibilizado ao fim do texto.

A partir desta pesquisa, espera-se ter contribuído para uma compreensão mais aprofundada de processos ecológicos associados à exploração de recursos efêmeros, como os padrões de diversidade e ocupação ambiental, influência das variáveis ambientais na morfologia e os reflexos da competição na bionomia de diferentes espécies. Um dos eixos integradores priorizou a competição entre espécies nativas e invasoras, a fim de delinear cenários de diferentes complexidades decorrentes do estabelecimento de espécies exóticas no Brasil e, particularmente, na região Nordeste. Por fim, mas não menos importante, acredita-se que os resultados desta pesquisa – que dá continuidade e lança diretrizes para linhas de investigação construídas no Grupo de Pesquisa Insetos de Importância Forense ao longo dos últimos 10 anos – ajudam a consolidar o arcabouço teórico-metodológico da Entomologia Forense no Brasil.

CAPÍTUO 1

Análise da montagem de comunidades de Calliphoridae (Diptera) de importância forense ao longo de um gradiente longitudinal no Nordeste brasileiro

Rodrigo F R Carmo; José R B Souza; Simão D Vasconcelos

1. INTRODUÇÃO

Constituição orgânica de espécies intimamente ligadas funcionando como um superorganismo é uma definição dada por Clements (1916) para comunidade biológica. Porém, em um outro ponto de vista, Gleason (1926) menciona que comunidades são associações fortuitas de indivíduos de espécies diferentes agrupadas em um mesmo ambiente por similaridades fisiológicas. Do ponto de vista da montagem de comunidades há uma relação de associação entre espécies que coexistem de forma a maximizarem a utilização dos recursos, não havendo necessariamente interdependência nessa associação (MENEZES et al., 2016). Assim, a montagem de comunidades surge como um mecanismo para o entendimento dos processos ecológicos inerentes a organização e ocorrência de espécies em uma comunidade local, definindo seus padrões no espaço e tempo (KEDDY, 1992).

Dentre os principais processos na determinação da ocorrência de espécies em uma comunidade estão os filtros abióticos e a similaridade limitante. O primeiro funciona como um mecanismo determinístico de seleção de espécies com características similares que permitem seu estabelecimento sob condições abióticas específicas (KEDDY, 1992). Já o limite de similaridade assume que a coocorrência de espécies somente é possível se elas possuírem uma baixa sobreposição de nicho (MacARTHUR & LEVINS, 1967), uma vez que espécies com características similares tendem a diminuir a coocorrência dada a exclusão competitiva. Associados a esses fatores, processos como dispersão e colonização-extinção devem ser levados em consideração na constituição das comunidades locais, já que elevadas taxas de dispersão tendem a aumentar a abundância local de espécies, reduzindo a probabilidade de extinção por competidores dominantes ou predadores (KNEITEL & MILLER, 2003). Por outro lado, as dinâmicas de colonização-extinção regulam a história de formação das assembleias (ou comunidades), influenciando diretamente sua estrutura e composição (CHASE, 2003).

A composição das comunidades locais obedece às condições biogeoclimáticas a que estão sujeitas. Dessa forma, para cada local e circunstância, acredita-se que haja uma comunidade específica e um padrão de sucessão típico. Espécies de Calliphoridae (Diptera) são excelentes modelos nas análises de composição e estrutura de comunidades locais. Popularmente conhecidas como moscas varejeiras, as espécies de Calliphoridae compreendem cerca de 1.550 espécies pertencentes a 109 gêneros descritas para o mundo (THOMPSON, 2013). Para a região Neotropical um total de 99 espécies agrupadas em 29 gêneros são registradas (KOSMANN et al., 2013), das quais grande parte é encontrada na região Nordeste do brasil. Algumas espécies dessa família apresentam uma importante função ecológica no processo de ciclagem da matéria orgânica, por serem agentes decompositores diretos (CATTS & GOFF, 1992). Além disso, as espécies de Calliphoridae têm sido utilizadas como uma ferramenta confiável na estimativa do intervalo *post-mortem* mínimo (IPM mínimo) uma vez que são pioneiras na detecção e colonização da carcaça ou cadáver (CATTS & GOFF, 1992; VASCONCELOS et al., 2013).

A fácil adaptação a diferentes ambientes de algumas espécies de Calliphoridae introduzidas no Brasil tem resultado no deslocamento de espécies nativas que compartilham nichos ecológicos semelhantes (GUIMARÃES et al., 1978). Acredita-se que nas últimas quatro décadas, a dinâmica populacional e a distribuição espacial das espécies de califorídeos no território brasileiro tenham sido afetadas pelo estabelecimento de espécies do gênero *Chrysomya* Robineau-Desvoidy, 1830 associada a ações antrópicas (MELLO et al., 2007). Carmo e Vasconcelos (2016) acreditam que em pouco mais de 40 anos passados o processo de invasão do gênero nas Américas, *C. megacephala* possa estar relacionada com o decréscimo populacional de *Cochliomyia macellaria* (espécie nativa) nesse ambiente. O efeito cascata da presença de espécies invasoras extrapola a comunidade de insetos, podendo afetar interconexões tróficas e ainda alterar propriedades básicas dos ecossistemas (WAGNER & VAN DRIESCH, 2010).

Com o estabelecimento de espécies necrófagas invasoras, a dinâmica da colonização dos substratos é alterada e por consequência a aplicação desses insetos na entomologia forense. Não somente na estimativa do IPM mínimo, mas também na potencial utilização de espécies como indicadoras de local de morte. Nesse cenário o principal objetivo do trabalho foi analisar os fatores estruturais da montagem das comunidades locais de Calliphoridae (Diptera) ao longo de diferentes paisagens fitofisionômicas do Nordeste brasileiro, avaliando o grau de sobreposição das espécies nativas e exóticas. De acordo com esses objetivos nos testamos as seguintes hipóteses: a) dada as diferentes condições biogeoclimáticas de cada fisionomia, haverá uma forte segregação das assembleias oriundas das extremidades do gradiente; b) a elevada plasticidade ambiental das espécies exóticas do gênero *Chrysomya* oferece uma maior distribuição ambiental frente as espécies nativas.

2. METODOLOGIA

2.1. ÁREA DE ESTUDO

Os estudos de campo foram realizados ao longo de um gradiente longitudinal no Nordeste brasileiro englobando diferentes arquiteturas de paisagens (ou fisionomias ambientais). Ao total 15 fisionomias foram amostradas partindo desde Fernando de Noronha (ilha oceânica) até Petrolina (Caatinga hiperxerófila arbórea-arbustiva) (Figura 1). Parte dos dados foram coletados por estudos prévios do Grupo de Pesquisa de Insetos de Importância Forense da UFPE (Carmo & Vasconcelos 2016, Soares 2014, Vasconcelos et al. 2015). O estado de Pernambuco foi utilizado com *proxy* das fitofisionomias encontradas no Nordeste brasileiro e suas características são encontradas na Tabela 1.

2.2. COLETA E IDENTIFICAÇÃO DOS INSETOS

Para a captura dos insetos foram utilizadas armadilhas suspensas a 80 cm do solo (Oliveira et al., 2016), iscadas a base de baço bovino (150 g) em estado inicial de decomposição (24h a 25°C). Em cada ambiente foram instaladas 10 armadilhas distantes 20 m uma das outras. Cada armadilha representa uma unidade amostral independente, portanto sendo classificada como uma réplica espacial para cada ambiente amostrado. As armadilhas ficaram expostas no ambiente por 48 horas. Os espécimes capturados foram sacrificados com acetato de etila e fixados em etanol a 70%, e então levados ao Laboratório de Insetos de Importância Forense da UFPE para posterior triagem e identificação. O presente trabalho teve foco direcionado a identificação das espécies pertencentes a família Calliphoridae. A identificação dos espécimes se deu através do uso da chave taxonômica específica (KOSMANN et al. 2013).

2.3. ANÁLISE DE DADOS

As análises de dados foram realizadas de modo a caracterizar as assembleias de Calliphoridae ao longo do gradiente, e em seguida de compará-las entre si. Dessa forma, as assembleias de califorídeos foram caracterizadas levando-se em consideração riqueza e composição de espécies, além de suas abundâncias absolutas e relativa. Como atributos assessórios na caracterização foram analisados a diversidade e dominância, além da equitabilidade.

A diversidade de espécies foi calculada através da série numérica de Hill (1973) sumarizada em um perfil de diversidade. O perfil de diversidade de Hill é composto por uma família de índices de diversidade matematicamente unificada (que diferem entre si apenas por um expoente q), os quais incorporam a abundância relativa e a riqueza de espécies (CHAO et al., 2014). Dentre as vantagens na utilização do perfil de diversidade de Hill está a facilidade comparativa dos valores numéricos, uma vez que os números de Hill são expressos em unidades de *números de espécies efetivas*. Ou seja, o número de espécies igualmente abundantes que seriam necessárias para dar o mesmo valor de uma medida de diversidade. Em suma, o perfil de diversidade de Hill engloba os valores de riqueza de espécies (${}^{0}D$), os valores do índice de entropia de Shannon (${}^{1}D$), além do índice de Simpson (${}^{2}D$), os quais servem como parâmetros comparativos entre as comunidades testadas.

O índice de dominância de Simpson foi usado como modelo analítico da dominância de espécies em cada assembleia, assessorado por um ranking de dominância construído a partir das abundâncias relativas das espécies em ordem decrescente. No ranking, quanto maior a distância entre as espécies maior é o grau de dominância. Esse gráfico auxilia visualmente a interpretação numérica do índice de dominância de Simpson. A equitabilidade foi calculada a partir da medida de uniformidade de Shannon também conhecida como equitabilidade de Pielou (J) – razão entre a diversidade observada e a diversidade máxima possível para aquele ambiente (MAGURRAN, 1988).

Para avaliar a distribuição das abundâncias de cada espécie nos ambientes testados foi realizada a Análise de Variância não paramétrica de Kruskal-Wallis (H). Os dados de abundância por ambiente foram transformados, em raiz quadrada, para a obtenção da matriz de similaridade e a partir do índice de Bray-Curtis foi realizada o escalonamento multidimensional não paramétrico nMDS para avaliar o quão similar são as áreas amostradas, seguida da análise de similaridade ANOSIM. Além disso, a diferença das assembleias foi acessada através da PERMANOVA, com seguida pelo teste *pair-wise*. Aliado a isso a diversidade beta para múltiplos locais, foi calculada levando-se em consideração a diversidade beta total através do índice de Sorensen (β_{SOR}), além de sua partição em *turnover* espacial pelo índice de dissimilaridade de Simpson (β_{SIM}) e o componente de aninhamento (β_{NES}) (Baselga *et al.*, 2013). As análises foram realizadas nas plataformas estatísticas Rstudio, PRIMER® 6.0 e STATISTICA® 7.0 ($\alpha < 0.05$).

3. RESULTADOS

Ao serem combinadas todas as 150 unidades amostrais, um total de 33.834 dípteros adultos distribuídos em 10 espécies da Calliphoridae foram registrados (Tabela 2). Os ambientes IOC e CHEa apresentaram os maiores valores de abundância (37,5% do total de indivíduos amostrados), o que conferiu uma diferença significativa em relação às demais áreas amostrais (Pseudo-F = 59,493; g.l. = 14;133; $P < 0{,}001$). A elevada abundância na Ilha Oceânica (IOC) não foi acompanhada pela riqueza, com somente 3 espécies (Tabela 2). Isso conferiu ao ambiente baixos valores de diversidade α (Figura 2). Por outro lado, Mata atlântica (MAT) foi a fisionomia que apresentou os maiores valores de diversidade α independente do expoente (q) analisado (Figura 2). Mata Atlântica urbana (MATu) e Caatinga Hipoxerófila (CHO) aparecem em seguida como as espécies mais diversas segundo a ordem de riqueza ${}^{0}D$ (7 e 6, respectivamente). Os perfis que apresentaram, de uma forma geral, os menores valores de diversidade foram as Monoculturas (MOM e MOC), além da Ilha Continental (ICO) (Figura 2). A diversidade α de Calliphoridae para Pernambuco foi moderada (${}^{0}D = 5{,}333$; ${}^{1}D = 2{,}482$ ${}^{2}D = 2{,}063$).

Os perfis de diversidade demonstram haver um padrão de diversidade α (baixo) para as assembleias oriundas de MOM, MOC, ICO e ECO, reflexo da elevada dominância encontrada nesses ambientes ($D_{Simpson}$ = 0,733; 0,803; 0,809 e 0,608, respectivamente). Os rankings de dominância apontam um padrão de codominância da maioria das assembleias ao longo do gradiente longitudinal (Figura 3). Além da elevada dominância, Agro ecossistemas, Ilha Oceânica e Ecótono apresentaram os menores valores de equitabilidade (J_{ICO} = 0,233; J_{MOC} = 0,276; J_{MOM} = 0,346). De uma forma geral, a distribuição das abundâncias de Calliphoridae em cada assembleia foi equilibrada, com índice de equitabilidade médio de $J_{\approx}$ = 0,574 (Tabela 3).

A análise de escalonamento multidimensional (nMDS) demonstrou haver forte tendência de segregação de grupos de acordo com o ambiente (Figura 4), validado numericamente pelo ANOSIM (R_{global} = 0,914; $P < 0,001$). O eixo nMDS 1 evidencia uma segregação espacial geográfica, com as assembleias de IOC e CHEb apresentando os maiores valores de dissimilaridade (R = 1; $P < 0,001$). Já o eixo nMDS 2 demonstra haver uma segregação gradual dos ambientes mais úmidos aos mais xéricos (Figura 4). Nesse eixo, MAT e CHEb apresentam os maiores valores de dissimilaridade (R = 1; $P < 0,001$). Ao ponto que o Ecótono se encontra exatamente entre as duas assembleias. A PERMANOVA *pair-wise* foi suficiente para demonstrar diferença em relação a distribuição das abundâncias entre todas as fisionomias testadas (Tabela 4).

As espécies invasoras *Chrysomya albiceps* e *C. megacephala* foram as espécies mais comuns nesse estudo, e representaram juntas um total de 66,7% dos indivíduos amostrados, seguidas por *Cochliomyia macellaria* (24,9%). A distribuição da abundância das espécies invasoras diferiu ao longo dos ambientes com *C. albiceps* sendo mais abundante em CHEa (H = 112,369; g.l. = 14; $P < 0,001$), enquanto sua congênere apresentou os maiores valores de abundância em IOC (H = 134,358; g.l. = 14; $P < 0,001$) (Tabela 2). As espécies *Mesembrinella bicolor* e *M. bellardiana* foram exclusivas para os ambientes de Mata Atlântica (MAT e MATu). Além delas, também só foram amostradas nesses ambientes. Por fim, o índice de Sorensen aponta uma elevada β diversidade (β_{SOR} = 0,664), sendo mais explicada pelo *turnover* de espécies entre os ambientes (β_{SIM} = 0,445) do que pelo aninhamento (β_{NES} = 0,217).

4. DISCUSSÃO

Os resultados do presente estudo apontam para uma diferenciação das estruturas das assembleias de Calliphoridae ao longo de um gradiente ecológico (longitudinal) no Nordeste do Brasil. Tal estruturação diferenciada é caracterizada principalmente pela distribuição das abundâncias e riqueza de espécies presentes em cada ambiente. Ao serem analisadas todas as assembleias ao longo do gradiente longitudinal, um gradiente de humidade pode ser estabelecido como explicação na diferenciação das assembleias (Figura 4 – eixo nMDS2). E de uma forma mais grosseira, apresenta uma tendência de formação de três grupos, com ambientes mais úmidos formando um primeiro grupo (ambientes insulares e mata atlântica) e no outro extremo ambientes mais xéricos (caatingas). Entre eles encontram-se os ambientes com perturbação humana (agro ecossistemas e áreas urbanas). Características inerentes às espécies formadoras dessas assembleias – especificidade ambiental por intolerância a variações climáticas por exemplo – conferem a elas a diferenciação e, por conseguinte a segregação dos grupos. Ilha oceânica foi o ambiente que apresentou maior tendência de segregação das demais assembleias. Isso está associado à organização da assembleia desse ambiente, tipicamente controlada por dominância (BEGON et al., 2007), onde *C. megacephala* aparece como a espécie mais abundante

Uma comunidade controlada por dominância apresenta espécies com estratégias competitivas distintas, sendo possível estabelecer uma relação preditiva dos processos de sucessão local com uma ou duas espécies apresentando características que lhes conferem superioridade no processo de competição (BEGON et al., 2007). Isso está de acordo com Aguiar-Coelho & Milward-de-Azevedo (1995), os quais analisaram a relação de coexistência das espécies *C. megacephala* e *C macellaria* de forma experimental. Os autores sugerem estratégias de colonização do recurso diferenciadas para cada espécie, com *C. megacephala* sendo a espécie vencedora e influenciando negativamente a espécie nativa, a qual apresenta um abandono precoce do recurso, além de formação de adultos mais leves, menores e menos fecundos (QUEIROZ & MILWARD-DE-AZEVEDO, 1991).

A elevada quantidade de micro-habitats proporcionada pela heterogeneidade ambiental da Mata Atlântica confere a existência de um mecanismo equalizador da comunidade, o qual atua na diminuição das desigualdades da competição entre as espécies locais (CHESSON, 2010; MAYFIELD & LEVINE, 2010), favorecendo a coexistência. Além disso, um dos pressupostos da teoria da montagem de comunidades propõe que comunidades mais próximas geograficamente tendem a ser mais semelhantes entre si (HUBBELL, 2001). Situadas dentro dos limítrofes da Mata Atlântica pernambucana, as áreas amostrais de Ilha continental (Itamaracá) e Mata Atlântica (Recife) distam apenas 50 km entre si. Tal proximidade e a ausência de barreiras físicas reais que impeçam o fluxo de espécies entre as comunidades atuam como fator promotor da similaridade dessas assembleias.

O mecanismo de heterogeneidade ambiental não pode ser empregado para a estruturação das assembleias oriundas de agro ecossistemas. A monotonia ambiental desse habitat pode estar relacionada com a estruturação da assembleia local pela associação de dois fatores intrínsecos à montagem da comunidade: exclusão competitiva e filtros abióticos. O primeiro apresenta um mecanismo ecológico determinístico em que uma espécie com maior crescimento populacional – ou maior capacidade competitiva – pode excluir espécies que desempenham a mesma função nesse local (HARDIN, 1960). Já o segundo está relacionado com mecanismos determinísticos de seleção de espécies com características biológicas que permitem o seu estabelecimento em condições abióticas específicas (KEDDY, 1992).

Dentre as cinco espécies amostradas nesses ambientes, somente *C. idioidea* apresenta bionomia e história de vida desconhecida. No entanto é sabido que as espécies pertencentes ao gênero *Chrysomya*, bem como *C. macellaria* e *Lucilia eximia* apresentam hábitos necrófagos (SMITH, 1986). Para esses ambientes *C. albiceps* apresentou cerca de 85% da abundância local, sendo considerada espécie dominante. Tamanha superioridade numérica está associada ao seu elevado potencial biótico, depositando cerca de 230 ovos por fêmea (GABRE et al., 2005), além do comportamento predatório no terceiro instar larval (AGUIAR-COELHO & MILWARD-DE-AZEVEDO, 1995).

Um distinto grupo é formado pelos ambientes mias xéricos, com as caatingas hiperxerófilas apresentando elevado grau de similaridade, e formadas praticamente pela mesma composição de espécies. O padrão de distribuição das abundâncias é proporcionalmente equivalente aos outros ambientes, contudo, a presença expressiva de *C. idioidea* confere a essas assembleias características únicas, que podem estar relacionadas com a formação desse grupo. Além do filtro ambiental atuando como fator determinístico da montagem dessas comunidades, não se pode descartar conjuntos de processos estocásticos baseados principalmente em restrição a dispersão do indivíduo (HUBBELL, 2001). Associados aos filtros ambientais pode-se destacar a tolerância a variações climáticas abruptas encontradas nesses ambientes (umidade e principalmente temperatura) como fatores explicativos da presença mais acentuada de *C. idioidea* compondo a assembleia de espécies da Caatinga. Nesse caso a estruturação e montagem das comunidades não são baseadas apenas na diferenciação ecológica das espécies, mas também em processos estocásticos de dispersão, extinção e especiação, ao ponto que todos os indivíduos são considerados ecologicamente semelhantes (HUBBELL, 2001).

O padrão de baixa riqueza e elevada abundância é esperado para a família Calliphoridae. Embora tenha-se registrado poucas espécies (S=10), isso corresponde a quase 15% de todas as espécies registradas para todo o território brasileiro (KOSMANN et al., 2013). Isso está associado à característica reprodutiva dessa família, tipicamente r-estrategista com elevada fecundidade (GABRE, 2005) e com um rápido desenvolvimento larval (AGUIAR-COELHO & MILWARD-DE-AZEVEDO, 1995). Nossos resultados demonstram haver diferença na abundância e composição de espécies ao longo das fisionomias. Tal diferença na composição de espécies reflete, também, a peculiaridade de cada ambiente. Ilha oceânica foi o ambiente menos rico, com apenas três espécies, porém fisionomia com maior número de indivíduos, sendo *C. megacephala* responsável por cerca de 68% da abundância geral para esse ambiente. O mesmo padrão (de elevada abundância de Calliphoridae em ilha oceânica) foi encontrado por Carmo & Vasconcelos (2016). Efeitos como a heterogeneidade ambiental, fragmentação do habitat e alteração da paisagem afetam diretamente o padrão de riqueza e abundância local das espécies (DEBINSKI & HOLT 2000).

Além disso, o efeito da escala é um fator que deve ser levado em consideração na ecologia de califorídeos, uma vez que a distribuição das abundâncias e composição das espécies dependem diretamente do uso potencial da paisagem e a capacidade de dispersão de cada espécie (ZABALA et al., 2014). Isso pode explicar a baixa riqueza de espécies na ilha. Características intrínsecas a um organismo invasor conferem a *C. megacephala* uma excelente capacidade adaptativa a diferentes fitofisionomias, fator relacionado com a sua elevada abundância nesse ambiente, dado o seu grande potencial biótico. Em laboratório, fêmeas de *C. megacephala* puseram em média 223 ovos, dos quais 30% atingiram a fase adulta (GABRE et al., 2005), enquanto Goodbrod & Goff (1990) registraram uma taxa de emergência de 44%.

No contraponto a elevada plasticidade ambiental e forte presença das espécies invasora, *Chloroprocta idioidea* merece destaque nesse estudo, com uma moderada distribuição e irregularidade de frequência. Os maiores valores de abundância dessa espécie ocorreram nos ambientes mais xéricos. Tal presença é corroborada pelos poucos estudos existentes sobre moscas necrófagas em ambientes de Caatinga (ALVES et al., 2014; VASCONCELOS et al., 2016). A entomologia forense aparece como uma útil ferramenta aos peritos criminais na resolução de crimes violentos. O conhecimento sobre a biologia e distribuição dos insetos associados à carcaças e/ou cadáveres serve como instrumento de auxílio aos órgãos criminais, estando principalmente relacionados com o cálculo do intervalo *post-mortem* mínimo (IPM mínimo) (GOFF et al., 1988). O entendimento da composição das espécies colonizadoras e suas dinâmicas no processo de colonização, além dos padrões de distribuição associados a dados bionômicos das espécies funcionam como pontos cruciais para uma melhor precisão na estimativa do IPM, e para a potencial utilização desses insetos como indicadores de local de morte (GREENBERG, 1991).

A dinâmica da colonização pode seguir um padrão preditivo de sucessão onde, na maioria das vezes os califorídeos são pioneiros nesse processo (CATTS & GOFF, 1992; VASCONCELOS et al., 2013). Isso confere a essas espécies potencial na utilização como ferramenta da estimativa do intervalo pós-morte. Esse fator está relacionado com a capacidade de distribuição dessas espécies em diferentes habitats. Em recente estudo realizado no Sudeste brasileiro Cabrini et al. (2013) mencionam a aplicabilidade de indicação de espécies do gênero *Mesembrinella* como indicadores ambientais, sendo *M. bellaridana* amostrada apenas em áreas de mata conservada. A mesma ideia de bioindicação é dada primordialmente por Carvalho e Linhares (2001), porém com outro modelo – *Hemilucilia semidiaphana*. Assim, baseado na amplitude da distribuição, padrões de abundância e riqueza, além da biologia da espécie é possível estimar o local onde a morte ocorreu (SMITH, 1986).

Porém, a utilização dessas espécies como indicadores de local de morte deve ser revista com cautela. Uma vez que em baixas latitudes com climas onde não há estações bem definidas, as variações ambientais de temperatura e umidade – fatores inerentemente relacionados com a dinâmica da colonização – são mínimas. Nossos resultados apontam para uma tendência de sobreposição de habitats entre a maioria das espécies amostradas ao longo do gradiente ecológico, havendo um claro padrão de dominância do gênero *Chrysomya* em todos os ambientes amostrados.

Para este trabalho as espécies *Hemilucilia segmentaria*, *H. semidiaphana* e *Mesembrinella bicolor* foram restritas a áreas florestadas, apresentando cerca de 31% dos insetos adultos desse local, resultado que corrobora estudos prévios realizados no Brasil (CARVALHO & LINHARES, 2001; MOURA et al., 1997). Os mesmos autores mencionam a restrição na distribuição dessas espécies para áreas florestadas, apontando a utilização da carcaça animal por essas espécies apenas para oviposição. No entanto não levam em consideração aspectos ecológicos inerentes à dinâmica da colonização (competição e predação).

Por fim, a importância forense compartilhada entre a utilização das espécies de Calliphoridae como ferramentas na estimativa do intervalo pós-morte e como bioindicadoras de local de morte merecem pesos distintos. O pioneirismo de uma espécie na localização e na dinâmica de colonização de uma carcaça confere maior acurácia ao perito na estimativa do intervalo pós-morte, e assim sendo, a utilização de espécies como *C. albiceps*, *C. megacephala* e até mesmo *C. macellaria* e *L. eximia* é válida. No entanto, nosso modelo anuncia em escala reduzida uma certa ineficiência da utilização das espécies de Calliphoridae como indicadoras de local de morte, dada a grande sobreposição de habitat da maioria das espécies amostradas. Mesmo com a presença exclusiva de *M. bicolor* e das espécies de *Hemilucilia* nas regiões florestadas, sua utilização como bioindicadores merece mais atenção para estudos futuros que envolvam análises de processos de coexistência com espécies mais plásticas, como as do gênero *Chrysomya*. No entanto em uma escala mais ampla (a nível de País) provavelmente, a utilização de espécies da família Calliphoridae ainda seja viável, uma vez que com o aumento da latitude as fitofisionomias tendem a mudar, e com isso as conformações das assembleias sigam essa mesma direção.

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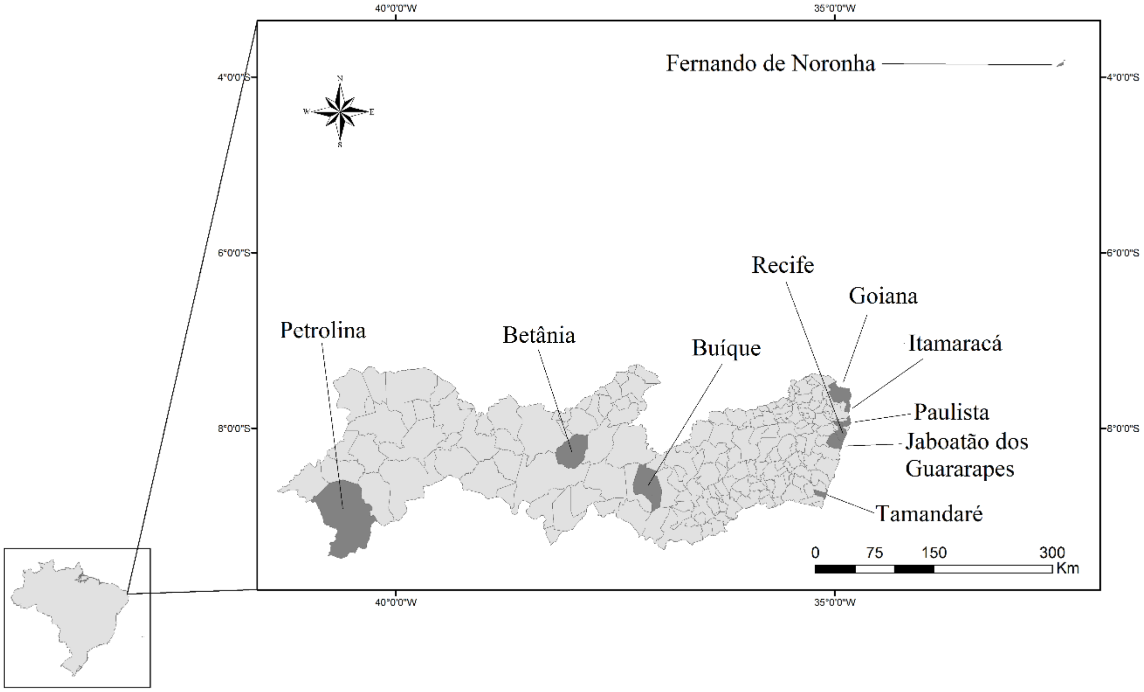
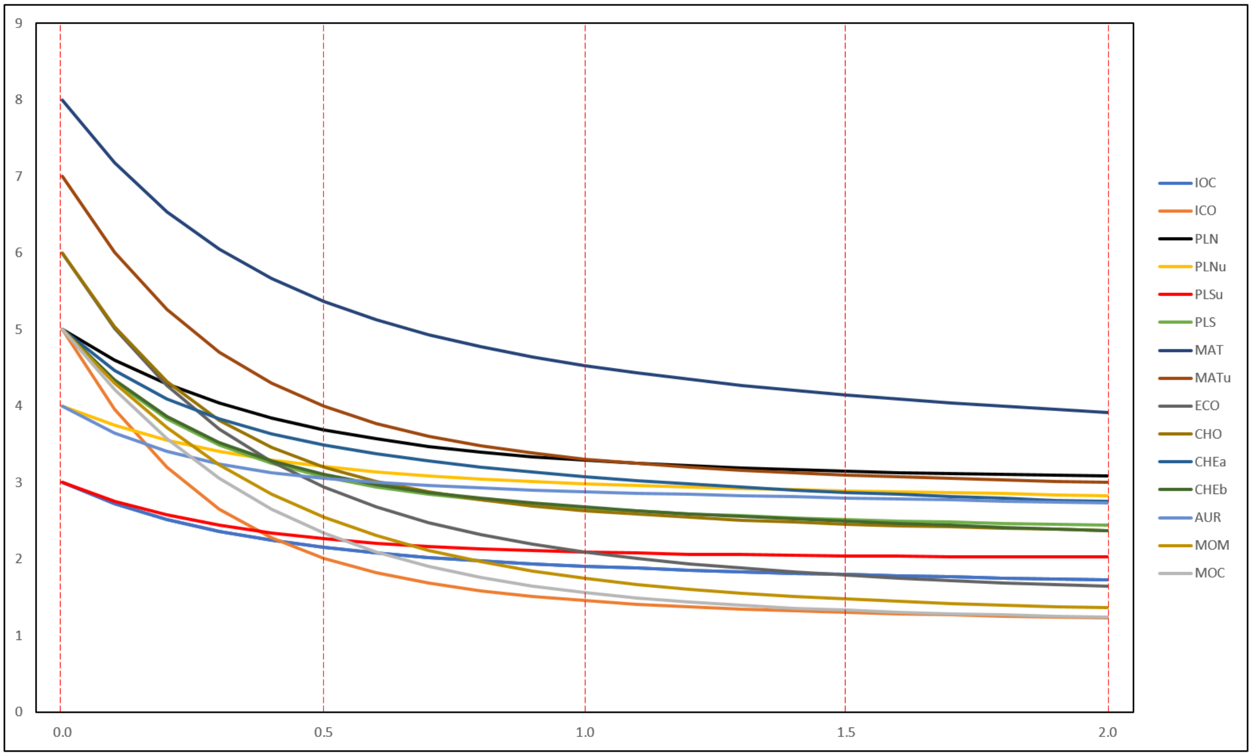
Figura 1. Mapa político de Pernambuco evidenciando as localidades de coleta (municípios).



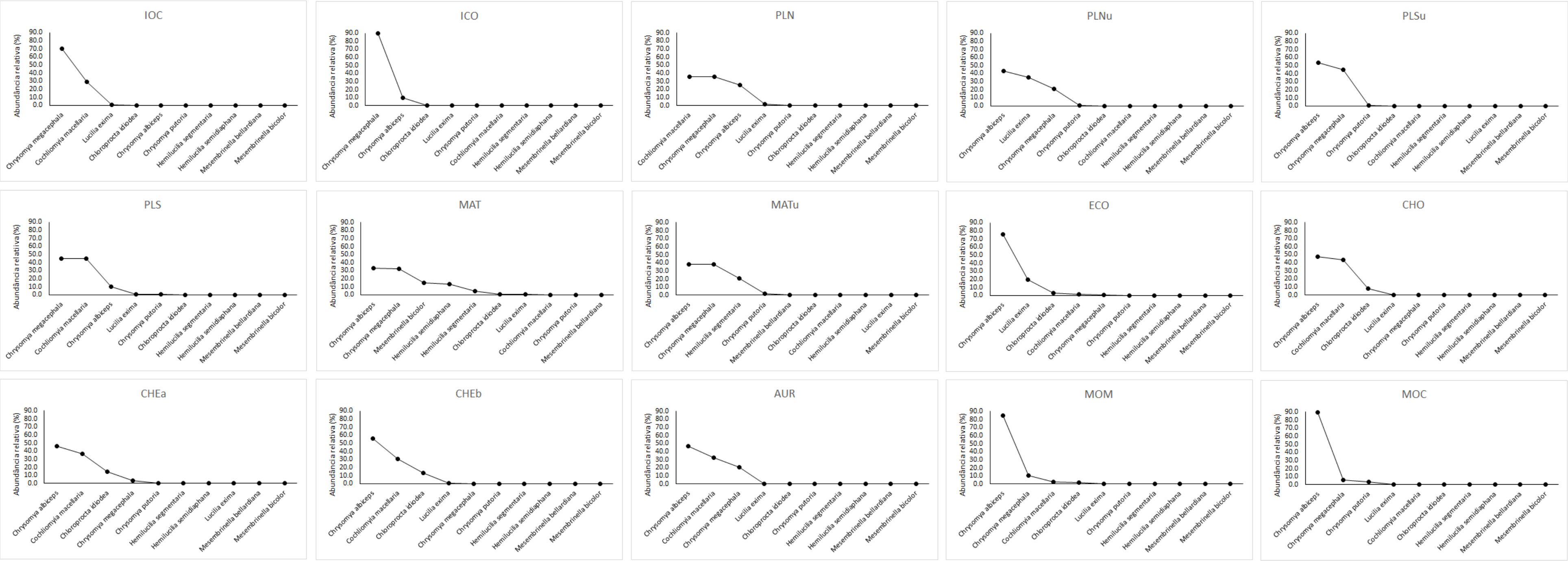
Fonte: O autor (2017).

Figura 2. Perfil de diversidade α seguindo a série de Hill (1973). IOC (Ilha Oceânica); ICO (Ilha Continental); PLN (Praia do Litoral Norte); PLNu (Praia do Litoral Norte urbana); PLSu (Praia do Litoral Sul urbana); PLS (Praia do Litoral Sul); MAT (Mata Atlântica); MATu (Mata Atlântica urbana); ECO (Ecótono); CHO (Caatinga Hipoxerófila); CHEa (Caatinga Hiperxerófila arbustiva); CHEb (Caatinga Hiperxerófila aerbóre-arbustiva); AUR (Área Urbana); MOM (Monocultura de Manga); MOC (Monocultura de Cana-de-açúcar).



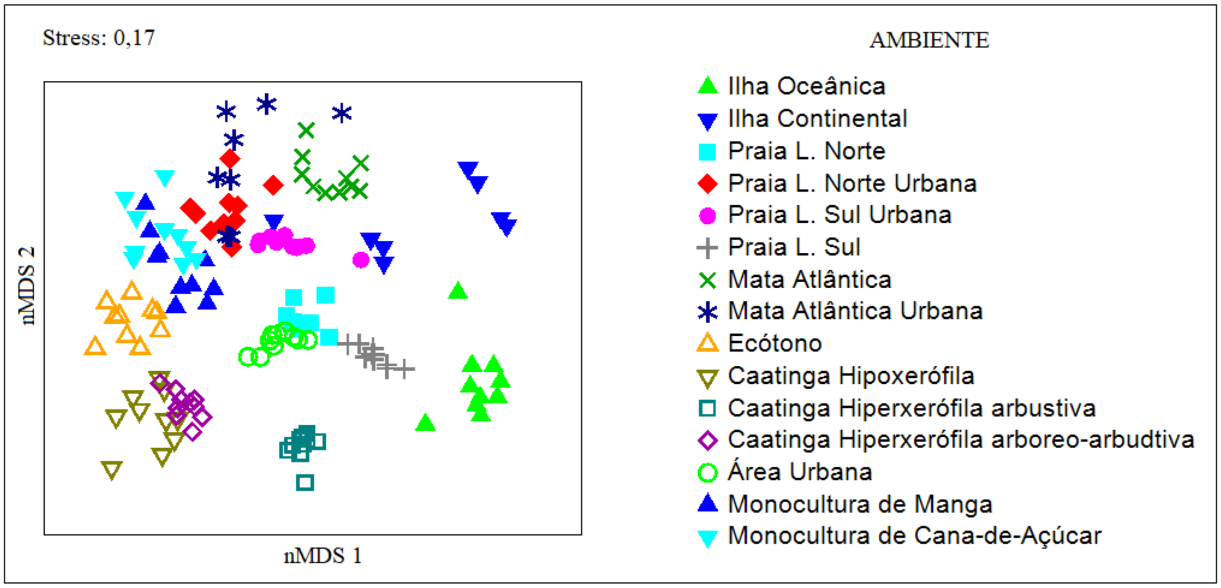
Fonte: O autor (2017).

Figura 3. Ranking de dominância das espécies de Calliphoridae ao longo do gradiente longitudinal de Pernambuco, Brasil. IOC (Ilha Oceânica); ICO (Ilha Continental); PLN (Praia do Litoral Norte); PLNu (Praia do Litoral Norte urbana); PLSu (Praia do Litoral Sul urbana); PLS (Praia do Litoral Sul); MAT (Mata Atlântica); MATu (Mata Atlântica urbana); ECO (Ecótono); CHO (Caatinga Hipoxerófila); CHEa (Caatinga Hiperxerófila arbustiva); CHEb (Caatinga Hiperxerófila aerbóre-arbustiva); AUR (Área Urbana); MOM (Monocultura de Manga); MOC (Monocultura de Cana-de-açúcar).



Fonte: O autor (2017)

Figura 4. Resultado da análise de escalonamento multidimensional não paramétrico (nMDS) oriundo da matriz de similaridade de Bray-Curtis para os ambientes amostrados em Pernambuco, Brasil.



Fonte: O autor (2017).

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Tabela 1. Localização dos locais de coleta demonstrando suas respectivas fisionomias, características ambientais e vegetação predominante.

Latitude	Longitude	Fisionomia	Distância para Capital	Clima	Temperatura	Umidade Relativa	Precipitação	Vegetação Predominante
03°51'13" S	32°25'25" O	Ilha Oceânica	545 km	As	26,5°C	78%	1418 mm/ano	Decídua sazonal
07°44'52" S	34°49'33"O	Ilha Continental	45 km	As'	25,3°C	75%	1867 mm/ano	Floresta perenifólia e restinga
07°33'38" S	35°00'09" O	Praia Litoral Norte	68 km	As'	24,4°C	75%	1730 mm/ano	Floresta atlântica e manguezal
07°54'37" S	34°49'22" O	Praia Litoral Norte (urbano)	20 km	As'	25,8°C	78%	1819 mm/ano	Restinga
08°06'46" S	35°00'54" O	Praia Litoral Sul (urbano)	18 km	As'	26,7°C	75%	1780 mm/ano	Ausente (urbana)
08°45'36" S	35°06'18" O	Praia Litoral Sul	106 km	As'	24,4°C	70%	1730 mm/ano	Restinga e Coqueiral
08°04'14" S	34°57'46" O	Mata Atlântica	12 km	As'	25,5°C	86%	2200 mm/ano	Mata Atlântica
07°36'05" S	35°01'00" O	Mata Atlântica (urbana)	63 km	As	27,1°C	75%	1924 mm/ano	Mata Atlântica com matriz de cana-de-açúcar
08°16'58" S	35°58'33" O	Ecótono	135 km	BSh	20,1°C	75%	765 mm/ano	Caatinga hipoxerófila com inserção de mata atlântica
08°29'56" S	37°22'14" O	Caatinga hipoxerófila	280 km	BShs'	21,0 °C	65%	926 mm/ano	Caatinga hipoxerófila arbórea-arbustiva
08°16'29" S	38°02'03" O	Caatinga hiperxerófila arbustiva	393 km	Aw	27,2 °C	62%	557 mm/ano	Caatinga hiperxerófila arbustiva
09°32'39" S	40°30'35" O	Caatinga hiperxerófila arbórea-arbustiva	710 km	BSh	28,1	58%	577 mm/ano	Caatinga hiperxerófila arbóreo-arbustiva
09°32'39" S	40°30'35" O	Área Urbana	710 km	BSh	28,1	58%	577 mm/ano	Ausente (urbana)
09°32'39" S	40°30'35" O	Monocultura de Manga	710 km	BSh	28,1	58%	577 mm/ano	Manga
07°36'05" S	35°01'00" O	Monocultura de Cana-de-açucar	63 km	As	27,1°C	75%	1924 mm/ano	Matriz de cana-de-açucar

Fonte: O autor (2017)

Tabela 2. Composição de espécies de Calliphoridae (Diptera) ao longo das fisionomias amostradas no Nordeste brasileiro. IOC (Ilha Oceânica); ICO (Ilha Continental); PLN (Praia do Litoral Norte); PLNu (Praia do Litoral Norte urbana); PLSu (Praia do Litoral Sul urbana); PLS (Praia do Litoral Sul); MAT (Mata Atlântica); MATu (Mata Atlântica urbana); ECO (Ecótono); CHO (Caatinga Hipoxerófila); CHEa (Caatinga Hiperxerófila arbustiva); CHEb (Caatinga Hiperxerófila aerbóre-arbustiva); AUR (Área Urbana); MOM (Monocultura de Manga); MOC (Monocultura de Cana-de-açúcar).

Espécies	**IOC**	**ICO**	**PLN**	**PLNu**	**PLSu**	**PLS**	**MAT**	**MATu**	**ECO**	**CHO**	**CHEa**	**CHEb**	**AUR**	**MOM**	**MOC**	**Total**	**AR**
Chloroprocta idiodea	0	6	0	0	0	0	19	1	56	41	917	131	0	12	0	1.183	3,5
Chrysomya albiceps	0	192	405	448	1.004	579	609	166	1.490	237	2.899	559	878	596	548	10.610	31,4
Chrysomya megacephala	4.471	1.774	567	221	843	2.568	599	166	18	1	210	2	390	75	36	11.941	35,3
Chrysomya putoria	0	5	9	7	18	13	0	9	1	1	4	0	0	0	22	89	0,3
Cochliomyia macellaria	1.827	0	572	0	0	2.545	2	1	28	216	2.296	311	613	16	3	8.430	24,9
Hemilucilia segmentaria	0	0	0	0	0	0	91	92	0	0	0	0	0	0	0	183	0,5
Hemilucilia semidiaphana	0	0	0	0	0	0	251	0	0	0	0	0	0	0	0	251	0,7
Lucilia exima	52	6	31	365	0	14	8	0	380	2	0	3	3	3	4	871	2,6
Mesembrinella bellardiana	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	2	0,0
Mesembrinella bicolor	0	0	0	0	0	0	274	0	0	0	0	0	0	0	0	274	0,8
Abundância Total	6.350	1.983	1.584	1.041	1.865	5.719	1.853	437	1.973	498	6.326	1.006	1.884	702	613	33.834	100
Abundância Relativa	18,8	5,9	4,7	3,1	5,5	16,9	5,5	1,3	5,8	1,5	18,7	3,0	5,6	2,1	1,8	100	
Riqueza	3	5	5	4	3	5	8	7	6	6	5	5	4	5	5	10	

Fonte: O autor (2017)

Tabela 3. Índices de Equitabilidade de Pielou (J) e dominância de Simpson (D) para as fisionomias amostradas ao longo do gradiente longitudinal de Pernambuco.

Ambientes	**J**	**D**
Ilha Oceânica	0.587	0.579
Ilha Continental	0.234	0.810
Praia Litoral Norte	0.740	0.324
Praia Litoral Norte (urbano)	0.788	0.353
Praia Litoral Sul (urbano)	0.671	0.494
Praia Litoral Sul	0.609	0.410
Mata Atlântica	0.726	0.255
Mata Atlântica (urbana)	0.615	0.333
Ecótono	0.412	0.609
Caatinga hipoxerófila	0.540	0.421
Caatinga hiperxerófila arbustiva	0.698	0.364
Caatinga hiperxerófila arbórea-arbustiva	0.612	0.421
Área Urbana	0.763	0.366
Monocultura de Manga	0.346	0.733
Monocultura de Cana-de-açucar	0.276	0.804

Fonte: O autor (2017)

Tabela 4. Resultado da análise par-a-par da PERMANOVA de acordo com a distribuição das abundâncias das espécies de Calliphoridae (Diptera) ao longo das fisionomias amostradas.

Combinações	**t**	**P(perm)**	**Permutações**
Ilha Oceânica, Ilha Continental	4.388	0.001	992
Ilha Oceânica, Praia L. Norte	5.743	0.001	996
Ilha Oceânica, Praia L. Norte Urbana	8.438	0.001	995
Ilha Oceânica, Praia L. Sul Urbana	8.693	0.001	991
Ilha Oceânica, Praia L. Sul	3.449	0.001	990
Ilha Oceânica, Mata Atlântica	9.527	0.001	993
Ilha Oceânica, Mata Atlântica Urbana	7.468	0.001	990
Ilha Oceânica, Ecótono	10.634	0.001	994
Ilha Oceânica, Caatinga Hipoxerófila	9.650	0.001	995
Ilha Oceânica, Caatinga Hiperxerófila arbustiva	8.213	0.001	993
Ilha Oceânica, Caatinga Hiperxerófila arboreo-arbudtiva	9.456	0.001	996
Ilha Oceânica, Área Urbana	6.621	0.001	989
Ilha Oceânica, Monocultura de Manga	9.270	0.001	994
Ilha Oceânica, Monocultura de Cana-de-Açúcar	9.785	0.001	990
Ilha Continental, Praia L. Norte	4.576	0.001	994
Ilha Continental, Praia L. Norte Urbana	4.492	0.001	994
Ilha Continental, Praia L. Sul Urbana	3.318	0.001	994
Ilha Continental, Praia L. Sul	4.790	0.001	994
Ilha Continental, Mata Atlântica	5.358	0.001	992
Ilha Continental, Mata Atlântica Urbana	3.612	0.002	988
Ilha Continental, Ecótono	7.676	0.001	992
Ilha Continental, Caatinga Hipoxerófila	8.248	0.001	992
Ilha Continental, Caatinga Hiperxerófila arbustiva	8.275	0.001	996
Ilha Continental, Caatinga Hiperxerófila arboreo-arbudtiva	8.277	0.001	989
Ilha Continental, Área Urbana	5.812	0.001	993
Ilha Continental, Monocultura de Manga	5.464	0.001	994
Ilha Continental, Monocultura de Cana-de-Açúcar	5.868	0.001	994
Praia L. Norte, Praia L. Norte Urbana	5.706	0.001	992
Praia L. Norte, Praia L. Sul Urbana	7.541	0.001	991
Praia L. Norte, Praia L. Sul	7.800	0.001	994
Praia L. Norte, Mata Atlântica	11.116	0.001	997
Praia L. Norte, Mata Atlântica Urbana	5.929	0.001	992
Praia L. Norte, Ecótono	11.144	0.001	992
Praia L. Norte, Caatinga Hipoxerófila	9.221	0.001	993
Praia L. Norte, Caatinga Hiperxerófila arbustiva	13.992	0.001	991
Praia L. Norte, Caatinga Hiperxerófila arboreo-arbudtiva	8.302	0.001	997
Praia L. Norte, Área Urbana	4.306	0.001	995
Praia L. Norte, Monocultura de Manga	7.066	0.001	994
Praia L. Norte, Monocultura de Cana-de-Açúcar	7.233	0.001	991

Praia L. Norte Urbana, Praia L. Sul Urbana	4.908	0.001	995
Praia L. Norte Urbana, Praia L. Sul	9.268	0.001	995
Praia L. Norte Urbana, Mata Atlântica	7.576	0.001	994
Praia L. Norte Urbana, Mata Atlântica Urbana	3.444	0.001	989
Praia L. Norte Urbana, Ecótono	5.925	0.001	993
Praia L. Norte Urbana, Caatinga Hipoxerófila	9.182	0.001	993
Praia L. Norte Urbana, Caatinga Hiperxerófila arbustiva	11.160	0.001	991
Praia L. Norte Urbana, Caatinga Hiperxerófila arboreo-arbudtiva	8.605	0.001	988
Praia L. Norte Urbana, Área Urbana	6.937	0.001	994
Praia L. Norte Urbana, Monocultura de Manga	3.866	0.001	991
Praia L. Norte Urbana, Monocultura de Cana-de-Açúcar	3.929	0.001	995
Praia L. Sul Urbana, Praia L. Sul	10.272	0.001	996
Praia L. Sul Urbana, Mata Atlântica	8.556	0.001	994
Praia L. Sul Urbana, Mata Atlântica Urbana	4.395	0.001	988
Praia L. Sul Urbana, Ecótono	9.037	0.001	989
Praia L. Sul Urbana, Caatinga Hipoxerófila	12.677	0.001	995
Praia L. Sul Urbana, Caatinga Hiperxerófila arbustiva	13.822	0.001	994
Praia L. Sul Urbana, Caatinga Hiperxerófila arboreo-arbudtiva	11.040	0.001	993
Praia L. Sul Urbana, Área Urbana	7.537	0.001	993
Praia L. Sul Urbana, Monocultura de Manga	5.238	0.001	994
Praia L. Sul Urbana, Monocultura de Cana-de-Açúcar	4.881	0.001	993
Praia L. Sul, Mata Atlântica	13.562	0.001	994
Praia L. Sul, Mata Atlântica Urbana	8.634	0.001	982
Praia L. Sul, Ecótono	13.817	0.001	995
Praia L. Sul, Caatinga Hipoxerófila	13.193	0.001	995
Praia L. Sul, Caatinga Hiperxerófila arbustiva	12.370	0.001	993
Praia L. Sul, Caatinga Hiperxerófila arboreo-arbudtiva	11.759	0.001	995
Praia L. Sul, Área Urbana	8.986	0.001	994
Praia L. Sul, Monocultura de Manga	9.835	0.001	995
Praia L. Sul, Monocultura de Cana-de-Açúcar	9.750	0.001	992
Mata Atlântica, Mata Atlântica Urbana	7.075	0.001	988
Mata Atlântica, Ecótono	10.977	0.001	996
Mata Atlântica, Caatinga Hipoxerófila	12.897	0.001	991
Mata Atlântica, Caatinga Hiperxerófila arbustiva	16.077	0.001	993
Mata Atlântica, Caatinga Hiperxerófila arboreo-arbudtiva	12.113	0.001	994
Mata Atlântica, Área Urbana	11.451	0.001	994
Mata Atlântica, Monocultura de Manga	7.743	0.001	992
Mata Atlântica, Monocultura de Cana-de-Açúcar	8.452	0.001	993
Mata Atlântica Urbana, Ecótono	8.221	0.001	990
Mata Atlântica Urbana, Caatinga Hipoxerófila	7.990	0.001	991
Mata Atlântica Urbana, Caatinga Hiperxerófila arbustiva	9.843	0.001	981
Mata Atlântica Urbana, Caatinga Hiperxerófila arboreo-arbudtiva	8.509	0.001	992
Mata Atlântica Urbana, Área Urbana	6.699	0.001	989
Mata Atlântica Urbana, Monocultura de Manga	4.118	0.001	986
Mata Atlântica Urbana, Monocultura de Cana-de-Açúcar	3.845	0.001	986

Ecótono, Caatinga Hipoxerófila	8.912	0.001	995
Ecótono, Caatinga Hiperxerófila arbustiva	11.299	0.001	992
Ecótono, Caatinga Hiperxerófila arboreo-arbudtiva	7.316	0.001	995
Ecótono, Área Urbana	9.722	0.001	993
Ecótono, Monocultura de Manga	5.464	0.001	991
Ecótono, Monocultura de Cana-de-Açúcar	6.230	0.001	995
Caatinga Hipoxerófila, Caatinga Hiperxerófila arbustiva	13.176	0.001	991
Caatinga Hipoxerófila, Caatinga Hiperxerófila arboreo-arbudtiva	3.161	0.001	993
Caatinga Hipoxerófila, Área Urbana	9.063	0.001	993
Caatinga Hipoxerófila, Monocultura de Manga	6.380	0.001	992
Caatinga Hipoxerófila, Monocultura de Cana-de-Açúcar	7.198	0.001	991
Caatinga Hiperxerófila arbustiva, Caatinga Hiperxerófila arboreo-arbudtiva	10.590	0.001	994
Caatinga Hiperxerófila arbustiva, Área Urbana	11.080	0.001	995
Caatinga Hiperxerófila arbustiva, Monocultura de Manga	9.962	0.001	997
Caatinga Hiperxerófila arbustiva, Monocultura de Cana-de-Açúcar	10.714	0.001	993
Caatinga Hiperxerófila arboreo-arbudtiva, Área Urbana	6.923	0.001	991
Caatinga Hiperxerófila arboreo-arbudtiva, Monocultura de Manga	5.667	0.001	993
Caatinga Hiperxerófila arboreo-arbudtiva, Monocultura de Cana-de-Açúcar	6.650	0.001	996
Área Urbana, Monocultura de Manga	5.900	0.001	987
Área Urbana, Monocultura de Cana-de-Açúcar	7.007	0.001	993
Monocultura de Manga, Monocultura de Cana-de-Açúcar	2.231	0.005	993

Fonte: O autor (2017)

CAPÍTULO 2

Esse capítulo segue as normas para publicação da Acta Zoologica

Running title – Blow fly populations in different Brazilian landscapes

Wing morphometrics as a tool to differentiate populations of *Cochliomyia macellaria* and *Chrysomya megacephala* (Diptera: Calliphoridae) from different environments in Northeastern Brazil

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Carmo, R.F.R., Astúa, D., Vasconcelos, S.D. 2016. **Wing morphometrics as a tool to differentiate populations of *Cochliomyia macellaria* and *Chrysomya megacephala* (Diptera: Calliphoridae) from different environments in Northeastern Brazil**. – *Acta Zoologica* (Stockholm)

Diptera are a standard model system to study environmentally induced phenotypic changes. We measured variation and covariation in wing morphology in five populations of *Cochliomyia macellaria* and *Chrysomya megacephala* (Calliphoridae) from different landscapes in Northeastern Brazil to test for morphometric structure, the presence of sexual dimorphism and the role of biogeoclimatic variables in the variation of wing morphology. We used geometric morphometric methods to assess the sexual dimorphism and differences among the populations. We detected a significant difference between sexes for both species, with females having larger and wider wings than males. The climate of each landscape influenced differently the wing morphology for each species. For *C. macellaria,* altitude, thermal amplitude and rainfall were the most significant factors associated with the size and shape variation, while for *C. megacephala*, relative humidity, maximum temperature and altitude were related with the variation in wing morphology. For both species, two groups of wings were found, one that has small and narrow wings, and the other presenting large and wide wings. Our results showed a significant differentiation between populations for both species, but a transversal comparison reveals that *C. megacephala* does not seem to be affected that the same way as native species by climatic variations.

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Introduction

Some animals have the differentiation of landscape architecture as a challenge to survivorship, and the adaptive ability is key for a successful establishment in different ecological scenarios. Phenotypic plasticity represents a solution to this “ecological problem” (Debat and David 2001; Fucso and Minelli 2010) and, in this sense, experimental and theoretical works have demonstrated that population dynamics plays a major role in the morphological differentiation of interacting species (Fanara *et al.* 2004; Fordyce 2006).

Phenotypic plasticity is the ability of an organism to express different phenotypes under different ecological condition, as landscape variability and resource availability (Agrawal 2001). As a developmental response, plastic traits allow species to cope with environmental variability (temporal or spatial) enabling a fitness optimization (such as acclimatization) (Ghalambor *et al.* 2007). Because any environmentally induced plasticity places phenotypes into different selective regimes, the fitness consequence will determine whether the response is adaptive, in both the short and long run (Alves *et al.* 2016).

Variation in adult size in holometabolous insects depends mostly on growth during larval stages (Nijhout *et al.* 2014). Yet nutrition is not the sole important factor, as body size is also influenced by abiotic factors such as temperature, humidity and rainfall of different sites (Alves *et al.* 2016). Recently, the effects of environmental factors on the shape of organisms, as well as the interactions of these elements, have been extensively examined (e.g. Beldade and Brakefield 2002; Prieto and Dahners 2009), and temperature seems to have the strongest influence on insect body variation (Hoffmann and Shirriffs 2002).

Diptera are an excellent model for studying phenotypic changes determined by environmental factors. Several species of medical and veterinary importance, such as blow fly species (Family Calliphoridae), exhibit variations in fitness-related and morphometric traits (wing size and shape) that can be associated with local selective pressures, mainly thermal selection (Reeve *et al.* 2000; Hoffmann and Shirriffs 2002; Demirci *et al.* 2012; Kjaersgaard *et al.* 2013; Hidalgo *et al.* 2015). Some phenotypic alterations can be subtle and difficult to detect, and geometric morphometrics techniques can provide valuable information on phenotypic variability with the advantage of being a low-cost and rapid tool (Dujardin 2011).

Chrysomya megacephala (Fabricius 1794) is a blow fly species of forensic and medical importance that has been used in forensic investigations given its intimate association with animal corpses and human cadavers (Oliveira and Vasconcelos 2010). Originally restricted to the Old World, the species was accidentally introduced in the Neotropical region in the late 1970s (Guimarães *et al*. 1978) and it is now categorized as an invasive species in many countries in the American continent. A recent review on necrophagous Diptera in Northeastern Brazil indicates that *Chrysomya* species have spread along diverse ecosystems with varying floristic compositions (Vasconcelos and Araújo 2012). This species competes directly with *Cochliomyia macellaria* (Fabricius, 1775), the secondary screw worm. It is a native species, widely distributed and abundant in the New World, ranging from southern Canada to Argentina (Baumgartner and Greenberg 1985). Both species occur in Neotropical Region, but in the last years, *C. megacephala* has been demonstrated to dominate insect assemblages in several environments (Vasconcelos et al. 2015), a fact that may provoke the displacement of the native species. The outcome of this competition may be associated with physiological (i.e., great biotic potential) and morphological traits, revealed by their phenotypic plasticity.

We investigated the wing size and shape differentiation of these species in populations from different landscape architectures in Northeastern Brazil, using techniques of wing geometric morphometrics. Specifically, we aimed to: i) test the presence of sexual dimorphism in the species evidenced by variations in size and shape of wing; ii) verify the existence of morphometric interpopulational variations; and iii) evaluate the association of environmental variables with morphological changes. According to these objectives, we tested the following hypotheses: i) because of the biological function of females, their wings are larger than male wings, without variation in wing shape, for both species; ii) the differentiation of landscape is associated with morphological variation for both species, and iii) temperature is the main biogeoclimatic factor to influence wing morphological differentiation for both species.

Materials and methods

Source of specimens

We sampled populations of *Cochliomyia macellaria* and *Chrysomya megacephala* (30 males and 30 females of each species in each environment) in five landscape architectures in Northeastern Brazil, in Pernambuco State (Fig 1). The sampled localities included one oceanic island, *Fernando de Noronha*; one continental island with rainforest enclaves, *Itamaracá*; two fragments of seasonally dry forest – one with hipoxerophytic vegetation, located in the municipality of *Buíque*, and the other characterized as hiperxerophytic vegetation, located in the municipality of *Betânia*; and one agroecosystem located at the municipality of *Petrolina*.

Fernando de Noronha is an oceanic island (3°51′13″S, 32°25′25″W), located 345 km from the Brazilian coast. It is legally protected, and economic activities (agriculture, tourism, commerce and industry) are limited or prohibited (Freitas and Vasconcelos 2008). With tropical climate, the island has an average annual precipitation of 1,418 mm and average temperature of 27.0 °C. The flora is poor, with mainly seasonal deciduous vegetation, and most plant species are exotic. *Itamaracá* is a continental island (07°44'52"S, 34°49'33"W) located 1.5 km offshore from the Brazilian coast with average annual precipitation and temperature 1,867 mm/year and 25.3 °C respectively (IBGE 2013). Approximately 77% of its area is urbanized, with the presence of housing, commerce and subsistence agriculture, Vegetation consists mostly of *restinga* evergreen forests and enclaves of rainfall with sparsely distributed mangrove areas.

Buíque (8°37′24″S, 37°9′23″W) houses part of the Catimbau National Park, a conservation unit designed to protect the fauna and flora of the native seasonally dry forest known as Caatinga. Its vegetation consists mostly of sub-deciduous and deciduous forest and the area is under maximum environmental protection by the Brazilian law. Climate is semiarid with average annual temperature of 21.0 °C and precipitation of 926 mm/year. The municipality of *Betânia* (08º16'29"S, 38º02'03"W) is located 348 km from the coast and its vegetation is categorized as hiperxerophytic *caatinga* – a dry forest composed of xeric shrub species, with the predominance of Cactaceae and sparsely distributed trees (e.g., Fabaceae, Anacardiaceae). The climate is semiarid with average annual precipitation and temperature of 432 mm and 26 °C, respectively. *Petrolina* (09º23'55"S, 40º30'03"O), is located 712 km from the coast, and it has a typical semiarid climate, with an average annual precipitation of 557 mm and temperature of 27 °C It is the largest agricultural site in Northeastern Brazil and a great part of its native xeric vegetation has been replaced with crops such as grape and melon.

Sampling expeditions took place between 2012 and 2015, always in the rainy season. We collected the flies using traps described by Vasconcelos et al. (2015) containing decomposing bovine spleen (200 g) as baits. In each environment, we installed six traps separated 20 m from each other, which were exposed in the field for 48 h. Insects were kept in 70% ethanol and identified using the taxonomical keys of Kosmann *et al.* (2013).

Environmental variables

Daily climatic data (rainfall, maximum temperature, minimum temperature, average temperature, thermal amplitude, relative humidity and wind speed) and altitude for all sites were obtained from the nearest meteorological stations (PCD 32564; OMM: 82900; OMM: 82890; OMM: 82886; OMM: 82983) of the *Instituto Nacional de Meteorologia*-INMET, Brazil and *Agência Pernambucana de Águas e Clima*-APAC, Brazil.. Data used corresponded to three months prior to collection. All variables were standardized (normalized) before the analysis.

Morphometric data acquisition

We mounted the wings on microscope slides. To avoid intra-individual variation interference, we only photographed the right wing, under 16 x magnification provided by a stereomicroscope Zeiss® (Stemi DV4). Nineteen wing type I landmarks of each species were digitized. That setup includes 15 landmarks proposed by Lyra *et al.* (2010) in addiction to 4 new landmarks (Fig 2). We tested the repeatability of coordinates (x, y) by MANOVA with 10 females selected randomly, digitized twice by the same person (RFRC). All landmarks were digitized in tpsDig2 version 2.16, after which the landmark configurations were superimposed using generalized Procrustes analysis – GPA (Rohlf and Slice 1990) in MorphoJ®. This method consists in three main steps. Firstly, wing configurations are translated to eliminate the position effect; secondly, landmark configurations are scaled to unit centroid size – defined as the square root of the sum of squared distance of landmarks from the center of gravity of a configuration (Bookstein 1991) – thus eliminating the effect of size. Finally, the superimposed configurations are rotated around the origin to minimize the summed square distance between homologous landmarks, which removes the effect of orientation.

Analysis of sexual dimorphism and differentiation of populations

To evaluate differences in wing size between the sexes for each species, Centroid Sizes (CS) were compared through factorial Analysis of Variance (ANOVA) using the software PAST®. We assessed shape differences between sexes by Discriminant Analysis (Mahalanobis distance) in MorphoJ®.

To test for differences in wing size between populations we compared CS using analyses of variance (ANOVA) in PAST software followed by Tukey pair-wise comparison tests. The relationship between CS and the environmental variables was assessed using a Spearman correlation for the populations for which data were available using Bio-Env analyses followed by a PERMANOVA in Primer6®.

We performed a multivariate analysis of variance (MANOVA, in PAST software) on shape coordinates (Procrustes coordinates) to test whether the populations diverged in shape. The difference among groups was characterized by discriminant function analyses and the reliability of group memberships was determined by cross-validation. The populations were ordered in a reduced space using canonical variable analysis (CVA) by MorphoJ® software. CVA is a technique that maximizes the separation of populations defined *a priori*, because each axis is constrained to represent the maximum between-group variance in relation to within-group variance. To assess the degree of correlation between size and shape (analysis of allometry), we fit a linear regression between the Procrustes coordinates and the CS. Lastly, we tested for an effect of biogeoclimate on phenotypic changes using a two-block partial least squares (PLS) analysis through PAST software. The PLS analysis tested for covariation between blocks of variables (wing shape within populations) using the Procrustes coordinates and environmental variables.

Results

Repeatability and analysis of sexual dimorphism

Comparison of two digitized sets for the same specimens showed a good repeatability for x, y coordinates, ranging from $R = 0.84$ to $R = 1.00$ ($R \approx 0.95$), even for Centroid Size ($R \approx 0.99$). Both species were found to be sexually dimorphic in wing size. The wings of females of *C. macellaria* and *C. megacephala* were larger than those of males (Table 1). *Cochliomyia macellaria* presented a significant difference in the shape of wings with landmarks 4, 5 and 6 towards the center of the wing, and 9 and 10 for the costal margin (Mahalanobis distance =

3.243; $P < 0.0001$), while for *C. megacephala* only the landmarks 9 and 10 showed displacement in the direction of the margin of the wing (Mahalanobis distance = 3.093; $P < 0.0001$).

Analysis of differentiation of populations through size and shape measurements

Wing size of both species varied differently among populations. Wings of female ($F_{3;117}$ = 1,625.2; $P < 0.0001$) and male ($F_{3;117}$ = 173.15; $P < 0.0001$) *C. megacephala* were smaller in Itamaracá populations ($P < 0.0001$) when compared with the other environments (Fig 3 and Table 2). On the other hand, for *C. macellaria*, wings from the populations of Fernando de Noronha had the smallest centroid size (Table 3), with the population of Petrolina presenting higher CS values (Fig 3; $F_{3;117}$ = 44.71; $P < 0.0001$ female, $F_{3;117}$ = 62.68; $P < 0.0001$ male).

Wing shape differed among all populations for both species (Table 4). The ordination of populations by CVA showed that the first and second canonical variable jointly explained approximately 80% (female) and 86% (male) of variability for *C. macellaria* and 85 % (female) and 91% (male) for *C. megacephala*. The first CVA axis separates the population of *C. macellaria* from Fernando de Noronha to the remainder (Fig 4). Females of this population had certain landmarks of the costal margin (landmarks 1, 2, 3 and 4) displaced in direction of the wing tip. Additionally, landmarks 9 and 10 were displaced towards the posterior region of the wing. For males, landmarks 1, 2, 3, 4, and 19 were displaced toward the center of the wing. The second canonical axis showed only local variations (Fig 4). These displacements denote wider wings in *C. macellaria* from Betânia. The cross-validation tests indicated that 93.7% (ranging between 81% and 99%) of individuals were correctly allocated to their respective populations (Table 5).

For *C. megacephala*, the firs axis demonstrated an isolation of Betânia population, more evident in the males (Fig 5). The landmarks 4, 7, 8, 9, and 10 for females and landmarks 5 and 6 for males were displaced toward the center of the wing. The second axis showed for females the same variation for the first one, with some punctual displacement (landmarks 11 and 12) to the margin of the wing. For males of this species, besides the landmarks 5 and 6, the CV2 showed the displacement of landmarks 4, 7, 9, and 10 toward the center of the wing (Fig 5).

Discriminant Analysis by Mahalanobis Distance for females of *C. megacephala*, could discriminate the isolation of population from Fernando de Noronha, with a great trend of marginal landmarks displaced to the center of the wing when compared with the other populations. This shape differentiation is evident between population from Fernando de Noronha and Betânia (P = 0.0272). The same pattern was observed for males of this species, with the addition of Betânia-Petrolina (P = 0.0024) (Table 6). The population of *C. macellaria* from Betânia showed landmarks 1, 2, 3 and 4 displaced to the margin of the wing, with no difference from the Petrolina population (P = 0.2859 – females). The males of this species presented similarity on the shape only between the populations from Buique and Petrolina (P = 0.2535 – Table 7). Furthermore, only females of *C. megacephala* showed allometric component in variation, with a weak but significant positive correlation between size and shape (r = 0.291, P < 0.0001).

Environmental factors

Bio-Env analyses, through Spearman correlation between CS and climatic variables, showed a weak relationship for both *C. macellaria* (Rho = 0.238, P < 0.001) and *C. megacephala* (Rho =

0.142, $P < 0.001$). Different variables influenced the distribution pattern of wing size in the species tested. Only average temperature did not have influence the distribution pattern of the CS for *C. macellaria*, while for *C. megacephala* the wing size was not affected by minimum temperature and wind speed (Table 8).

The PLS analyses showed that all dimensions were statistically significant in the explanation of the wing shape variation according to bio-geoclimatic variables ($P < 0.01$), with PLS1 axis explaining 85 – 89 % of the total variation for *C. macellaria*, and 77 – 79% for *C. megacephala*. Altitude, Maximum Temperature, Average Temperature and Thermal Amplitude were negatively correlated with the shape of *C. macellaria* wings, whereas Rainfall showed the highest positive correlation with PLS1 (Table 9). The same pattern of association was found when *C. megacephala* was analyzed (Table 10). The populations of *C. macellaria* were clustered in two groups along this axis with populations of Fernando de Noronha isolated from the others (Fig 6), while *C. megacephala* displayed a group formation with populations of Fernando de Noronha and Itamaracá (with positive values), and another with Betânia and Petrolina with negative values (Fig 7). Thus, lower elevations and high rainfall were associated with wider wings whilst greater elevations and low rainfall were associated with narrower wings (see results of CVA for both species).

Discussion

Sexual dimorphism

Our study elucidates sexual dimorphism in wing size for both *C. macellaria* and *C. megacephala*, with females larger than males irrespective of the population, in accordance with our initial hypothesis, and with the data from Lyra *et al*. (2010). Although larger females of species of the genus *Chrysomya* were previously described (Hu *et al*. 2011), no study had applied wing morphometrics to detect sexual dimorphism in *C. megacephala*. It is known that the size and potential fertility of blow flies reflect the comparative success of the larvae in obtaining food before pupation (Norris 1963). However, temperature is an important factor for predicting sexual dimorphism in blow flies, since body size may increase differently according to temperature variation, which is the case of *C. megacephala* (Hu *et al.* 2011). This seems to be the case of the populations investigated here, in which a more accentuated sexual dimorphism was evidenced in localities exposed to wider temperature variations.

Wing patterns are sex-specific and reflect the selective pressures endured by each sex. However, the hypothesis of sexual selection cannot be ruled out (Cator and Harrington 2011). Our results show a clear difference in wing shape between males and females for both species (females have narrower wings than males in both species). Some researchers suggest that possible causes for this differentiation include natural selection, with singular adaptation of each sex to different ecological niches (Shine 1989; Bonduriansky 2006). Although we did not test the correlation of functional significance with wing shape variation, differences in foraging habitats and reproductive behavior may influence the shape dimorphism observed, as proposed for other insect species (Sivinski and Dodson 1992; Bonduriansky 2006). Integrative studies involving not only ecological parameters of differences in foraging habitats and behavior but also reproductive activities and flight agility or resistance, with wing morphometric analyses may provide a better explanation for this differentiation.

Differentiation of populations through wing size and shape related to environmental factors

Landscape architecture acts as an important factor on differentiation of population structure in insects, influencing directly the morphological variability (Alves *et al*. 2016). Such premise is in accordance with our hypothesis, as all populations for both species differ in wing size and shape, but without correlation between the two variables. Variation in size between populations are commonly associated with alterations in shape (Dujardin 2008), as an allometric factor. We did not detect the effect of allometry in our study model, except for a weak correlation in wing shape and size of *C. megacephala* females This absence of correlation associated with geographical variations in wing shape has also been found to occur in other dipterans; *Drosophila serrata* (Malloch, 1927) (Hoffmann and Shirriffs 2002) and *Polietina orbitalis* (Stein, 1904) (Muscidae) (Alves *et al*. 2016). Lyra et al (2010) found the same pattern for *C. macellaria*, with only a small proportion (< 5%) of shape variation due to size. The absence of allometric component in our study highlights the need to investigate the influence of other factors (environmental) in the variability of wing shape, and mainly in wing size.

The climate associated with landscape of each environment influences differently wing size for both species. For *C. macellaria*, altitude, thermal amplitude and rainfall were the most significant factors associated with the wing size variation, while for *C. megacephala*, relative humidity, maximum temperature and altitude were related with the variation in wing size. The effect of altitude – factor in common with both species – has been reported in other studies with others insects as a model (Belen *et al.* 2004; Demirci *et al* 2012; Alves *et al* 2016). However, these studies analyzed only the relationship between altitude and wing shape, revealing that in high altitudes insects with narrower wings are found. In this context, our data support a similar pattern, as wider wings were associated with populations sampled in areas located at lower altitudes.

Although there is no allometric effect in the distribution of wing size and shape for both species in our study, it appears that the large and thin wing found in populations at higher elevations could confer an advantage for these flies. This can be explained because the reduction of air density at higher elevations can interfere with the aerodynamic forces related to flight (Dudley 2000). However, only in the native species the altitude could better explain the wing shape variability. Such environmental variable has low variation when compared with others (temperature and humidity), and can be considered a conservative attribute to explain the wing variation in *C. macellaria*. On the other hand, differences on *C. megacephala* wings (an invasive species) were better explained by temperature and humidity, factors that corroborate the wide plasticity of this species.

Moreover, food availability for the larvae ultimately affects the size of adults – which in turn influences wing size and shape. For example, food availability and larval competition influenced CS variation in adult mosquitoes (Schneider *et al.* 2004; Paaijmans *et al.* 2009; Kweka *et al.*2012). In necrophagous flies this multidisciplinary research line is not yet established. From that perspective, it is known that dry forests have, comparatively, a more limited availability of resources (i.e., carrion) when compared to rainforests and this could have influenced adult size of blow fly species.

In this study, we sampled oceanic islands (Fernando de Noronha), continental island (Itamaracá), hipoxerophytic Caatinga (Buíque), hiperxerophytic Caatinga (Betânia), and agroecosystem (Petrolina) and demonstrated that morphometric traits can vary according to the environment. We suggest that environmental parameters, such as temperature and relative humidity have indirect effects on the ecology of blow fly assemblages. For instance, carrion decomposition is strongly determined by local climatic characteristics (Byrd and Castner 2010) which are associated with food availability for necrophagous larvae.

Assemblages of necrophagous flies vary markedly in different types of environment in the Neotropical region (Vasconcelos *et al*. 2015), but a few studies have correlated species dominance with morphometric traits. We evidence here that environmental factors (e.g., altitude and temperature) and geographical isolation may have produced populations with unique wing patterns in oceanic islands (that is, Fernando de Noronha). This trend is observed for both native and invasive species, a fact that stimulates future studies on invasiveness and adaptation of necrophagous flies. Flies from this island had wider wings, in an inverse proportion with altitude.

Our results show a significant differentiation between populations for both species, but a transversal comparison reveal that *C. megacephala* does not seem to be affected in the same way as the native species by climatic variations in the field experiment. Temperature and humidity appear to be the most significant environmental variables associated with differences in wing size and shape for *C. megacephala* which denote a high capacity of this species to adapted to different environment. Furthermore, we believe that an integrative study involving knowledge from genotypic expression with influence of environment factors in the phenotypic plasticity of blow flies species, serve as a further tools to elucidate biological invasion.

Acknowledgements

We thank the following people and institutions: *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq), *Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco* (FACEPE) and *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) for financial support; André Esteves (UFPE), Ricardo Araújo (*Parque Nacional Marinho de Fernando de Noronha*), Reginaldo Valença (Fernando de Noronha administration) and José Alves Siqueira Filho (*Centro de Referência para Recuperação de Áreas Degradadas*), for logistical support; Diogo Albuquerque, Taynã Olimpia, Fernanda Santos, Leonardo Pereira, Marcelo Soares, Taciano Barbosa and Diego Oliveira for their invaluable help in insect sampling and identification. Insect collection was performed under authorization of the *Instituto Chico Mendes de Conservação de Biodiversidade* (ICMBio) - 32657-1.

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TABLES:

Table 1. Results of ANOVA on centroid size for *C. macellaria* (CM) and *Ch. megacephala* (CH) – d.f. = degree of freedom.

CM	**F**	***p***	**d.f.**
Local	92.4	< 0.001	3
Sex	11.1	< 0.005	1
Local x Sex	6.5	< 0.005	3
CH	**F**	***p***	**d.f.**
Local	9039.7	< 0.001	3
Sex	14.3	< 0.005	1
Local x Sex	2.5	0.061	3

Table 2. Post-hoc ANOVA test *Tukey* for *Ch. megacephala* according to Centroid Size. Significant values are in bold.

♂ ♀	Betania	F. Noronha	Itamaraca	Petrolina
Betania	-	**< 0.0001**	**< 0.0001**	0.0594
F. Noronha	**< 0.0001**	**-**	**< 0.0001**	0.9431
Itamaraca	**< 0.0001**	**< 0.0001**	-	**< 0.0001**
Petrolina	**< 0.0001**	0.9997	**< 0.0001**	**-**

Table 3. Post-hoc ANOVA test *Tukey* for *C. macellaria* according to Centroid Size. Significant values are in bold.

♂ ♀	Betania	F. Noronha	Itamaraca	Petrolina
Betania	-	0.7141	**< 0.0001**	**< 0.0001**
Buique	0.5828	-	**< 0.0001**	**< 0.0001**
F. Noronha	**< 0.0001**	**< 0.0001**	**-**	**< 0.0001**
Petrolina	**< 0.0001**	**< 0.0001**	**< 0.0001**	**-**

Table 4. Results of MANOVAs among different landscapes and wing shape of *C. macellaria* and *Ch. megacephala*.

Species	Wilk's lambda	*F* statistics	d.f. 1	d.f. 2	*p* value
C. macellaria - female	0.01935	5.622	114	234.6	< **0.0001**
C. macellaria - male	0.04143	3.998	114	240.5	< **0.0001**
Ch. megacephala - female	0,05316	3,510	114	240.5	< **0.0001**
Ch. megacephala - male	0.03549	4.214	114	234.6	< **0.0001**

Table 5. Percent correct classification of populations of *Cochliomyia macellaria* and *Chrysomya megacephala* based on wing morphology and discriminant function analysis.

Pupulation	***Cochliomyia macellaria***		***Chrysomya megacephala***	
	Female	**Male**	**Female**	**Male**
Fernando de Noronha	95	98	90	99
Itamaracá	n.a.	n.a.	90	81
Buíque	90	100	n.a.	n.a.
Betânia	90	99	99	99
Petrolina	95	95	95	85

Table 6. Mahalanobis distance – derived from Discriminant Analysis – among mean wing shape configuration of *Chrysomya megacephala* populations. Significant values after Bonferroni correction are in bold.

♂ ♀	Betânia	F. Noronha	Itamaracá	Petrolina
Betânia	-	**0.0330**	0.1861	**0.0024**
F. Noronha	**0.0272**	-	0.9981	0.2092
Itamaracá	0.2695	0.1902	-	0.3861
Petrolina	0.6452	0.3665	0.6806	-

Table 7. Mahalanobis distance – derived from Discriminant Analysis – among mean wing shape configuration of *Cochliomyia macellaria* populations. Above diagonal male, below diagonal female. Significant values after Bonferroni correction are in bold.

♂ ♀	Betânia	Buique	F. Noronha	Petrolina
Betânia	-	0.9252	**0.0105**	0.8868
Buique	0.1851	-	**0.0126**	0.9942
F. Noronha	**0.0024**	**0.0013**	-	**0.0134**
Petrolina	0.2859	0.2535	**0.0121**	-

Table 8. PERMANOVA results between the centroid size and environmental variables for both species.

Variables	*Cochliomyia macellaria*		*Chrysomya megacephala*	
	Pseudo-F	*p*	Pseudo-F	*p*
Altitude	102.36	**< 0.001**	60.25	**< 0.001**
Rainfall	82.18	**< 0.001**	59.16	**< 0.001**
Maximum Temperature	43.88	**< 0.001**	94.83	**< 0.001**
Minimum Temperature	75.21	**< 0.001**	2.86	0.089
Thermal Amplitude	89.29	**< 0.001**	35.94	**< 0.001**
Average Temperature	2.31	0.121	76.4	**< 0.001**
Relative Humidity	16.25	**< 0.001**	181.66	**< 0.001**
Wind Speed	39.33	**< 0.001**	1.33	0.253

Table 9. PLS result between the shape and environmental variables for *Cochliomyia macellaria*. Notice that the greater PLS1 has the strongest correlation (all $p < 0.01$).

Variables	Dimensions for Females							
	1	2	3	4	5	6	7	8
Altitude	-0.442	0.167	-0.068	-0.044	-0.120	0.468	-0.698	-0.226
Rainfall	0.429	0.017	-0.006	-0.205	0.780	-0.046	-0.402	-0.050
Maximum Temperature	-0.361	-0.231	-0.058	-0.034	0.030	-0.748	-0.173	-0.471
Minimum Temperature	0.343	-0.482	-0.372	0.478	-0.253	-0.069	-0.403	0.226
Thermal Amplitude	-0.422	0.058	0.138	-0.047	0.150	-0.279	-0.204	0.810
Temperature Average	-0.176	-0.525	-0.476	-0.616	0.050	0.212	0.173	0.098
Relative Humidity	0.140	0.638	-0.674	-0.158	-0.161	-0.250	-0.024	0.071
Wind Speed	0.380	-0.023	0.392	-0.565	-0.512	-0.171	-0.295	0.061
Singular values	3.905	1.266	0.340	0.210	0.192	0.119	0.070	0.047
Covariation (%)	89.34	9.38	0.68	0.26	0.22	0.08	0.03	0.01
Correlations	0.724	0.429	0.314	0.283	0.339	0.541	0.482	0.377
Variables	Dimensions for Males							
	1	2	3	4	5	6	7	8
Altitude	-0.380	0.259	-0.019	0.041	-0.056	0.448	0.725	-0.240
Rainfall	0.411	-0.031	-0.089	0.114	-0.866	-0.110	0.216	-0.008
Maximum Temperature	-0.422	-0.167	-0.056	-0.419	-0.152	-0.587	0.048	-0.495
Minimum Temperature	0.226	-0.552	-0.309	0.122	0.346	-0.267	0.555	0.188
Thermal Amplitude	-0.399	0.174	0.075	-0.218	-0.173	-0.239	0.144	0.806
Temperature Average	-0.315	-0.556	-0.418	-0.172	-0.240	0.501	-0.263	0.101
Relative Humidity	0.271	0.473	-0.706	-0.435	0.122	0.007	-0.013	0.010
Wind Speed	0.356	-0.191	0.464	-0.727	0.042	0.250	0.167	0.031
Singular values	3.778	1.355	0.690	0.231	0.178	0.113	0.046	0.036
Covariation (%)	85.54	11.00	2.86	0.32	0.19	0.08	0.01	0.01
Correlations	0.744	0.435	0.483	0.403	0.420	0.324	0.246	0.393

Table 10. PLS result between the shape and environmental variables for *Chrysomya megacephala*. Notice that the greater PLS1 has the strongest correlation (all $p < 0.01$).

Variables	Dimensions for Females							
	1	2	3	4	5	6	7	8
Altitude	-0.262	-0.302	0.399	-0.451	0.508	-0.025	0.461	-0.077
Rainfall	0.453	-0.080	-0.237	0.062	0.231	0.781	0.250	-0.054
Maximum Temperature	-0.363	-0.280	0.178	-0.068	-0.009	0.438	-0.481	0.575
Minimum Temperature	0.183	-0.552	0.243	0.448	0.258	-0.105	-0.389	-0.416
Thermal Amplitude	-0.455	0.193	-0.074	-0.246	-0.130	0.356	-0.262	-0.691
Temperature Average	-0.275	-0.351	0.153	0.363	-0.605	0.156	0.502	-0.058
Relative Humidity	0.381	0.336	0.797	-0.105	-0.250	0.159	-0.080	-0.046
Wind Speed	0.364	-0.496	-0.174	-0.619	-0.420	-0.105	-0.128	-0.065
Singular values	2.918	1.437	0.522	0.238	0.172	0.138	0.093	0.027
Covariation (%)	77.63	18.84	2.49	0.52	0.27	0.17	0.08	0.01
Correlations	0.673	0.451	0.358	0.360	0.351	0.306	0.288	0.230

Variables	Dimensions for Males							
	1	2	3	4	5	6	7	8
Altitude	-0.254	0.331	-0.292	0.386	-0.248	0.569	-0.455	-0.012
Rainfall	0.503	0.002	0.051	0.770	0.311	-0.214	-0.097	-0.011
Maximum Temperature	-0.294	0.360	-0.146	0.254	-0.088	-0.203	0.513	0.621
Minimum Temperature	0.302	0.521	-0.236	-0.176	0.334	0.328	0.450	-0.360
Thermal Amplitude	-0.467	-0.065	0.007	0.342	-0.193	-0.252	0.306	-0.683
Temperature Average	-0.207	0.443	-0.246	-0.198	0.319	-0.578	-0.467	-0.089
Relative Humidity	0.199	-0.386	-0.872	-0.032	-0.169	-0.132	0.067	0.002
Wind Speed	0.452	0.375	0.120	-0.082	-0.745	-0.259	-0.042	-0.097
Singular values	3.035	1.649	0.506	0.311	0.199	0.173	0.081	0.030
Covariation (%)	74.52	22.00	2.07	0.78	0.32	0.24	0.05	0.01
Correlations	0.601	0.424	0.454	0.389	0.414	0.296	0.245	0.350

LIST OF FIGURES:

Figure 1. Location of sample site along of eco-regional gradient in Pernambuco State – Brazil

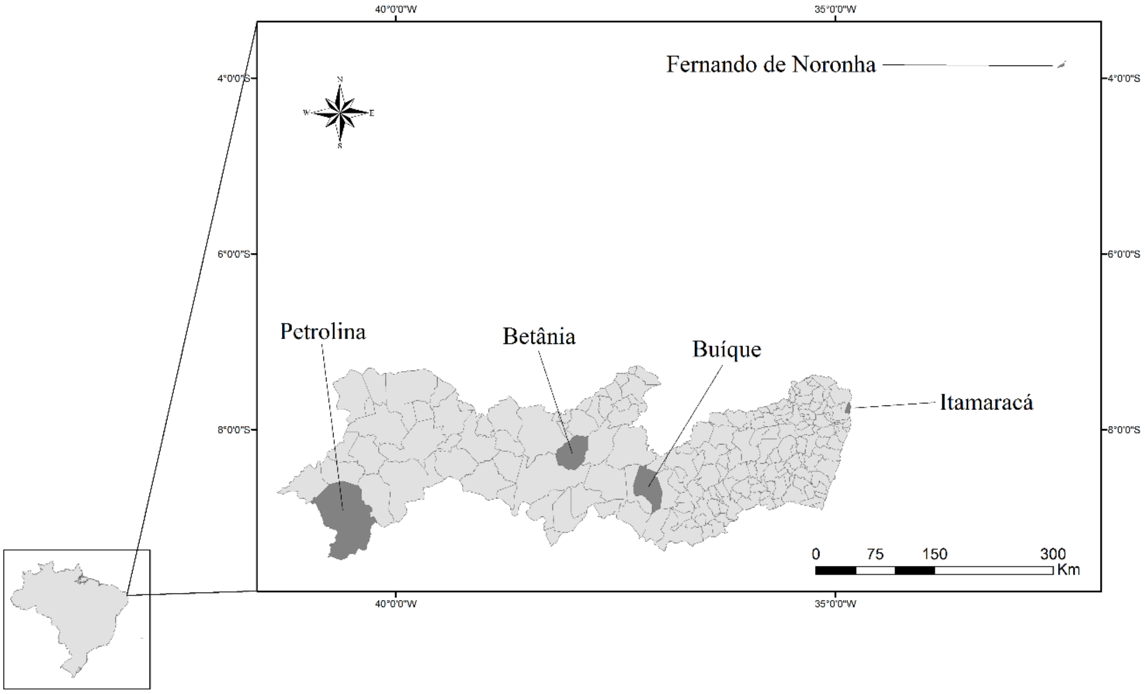


Figure 2. Wing of *Chrysomya megacephala* (left) and *Cochliomyia macellaria* (right) showing the 19 landmarks used in the morphometric analyses.

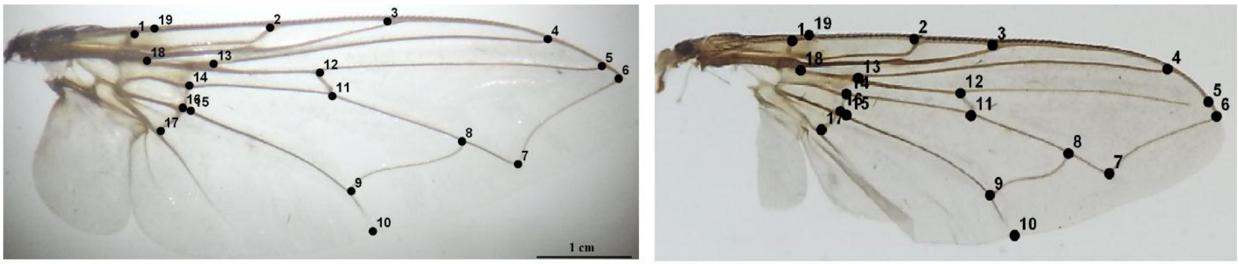


Figure 3. Average of the Centroid Size for *Cochliomyia macellaria* (a) and *Chrysomya megacephala* (b) along to different sample sites: female in gray and male in black.

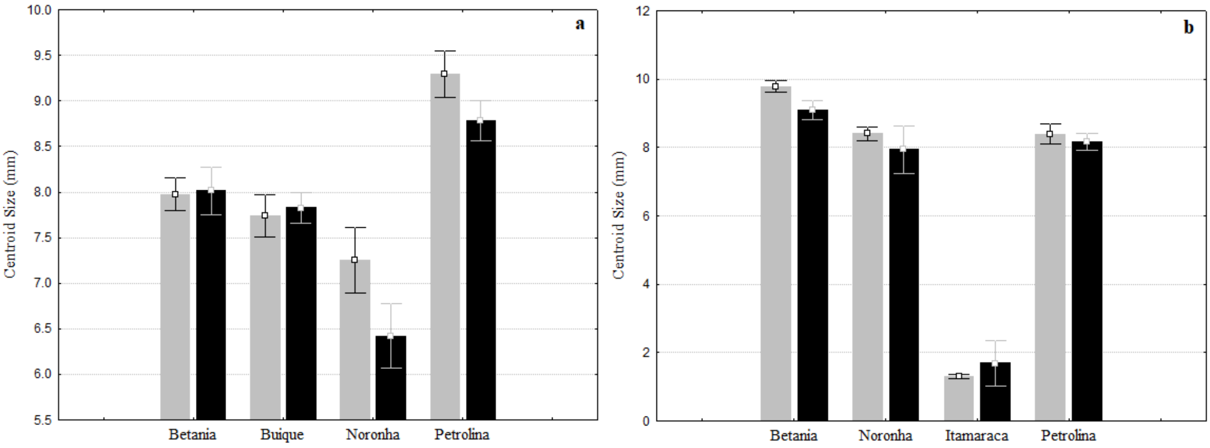


Figure 4. Canonical Variate Analysis (CVA) for wing shape of *Cochliomyia macellaria* (female – left, and male – right) and the transformation grid of the wing shape of individuals in each axis. The vectors indicate the direction of the deformation direction.

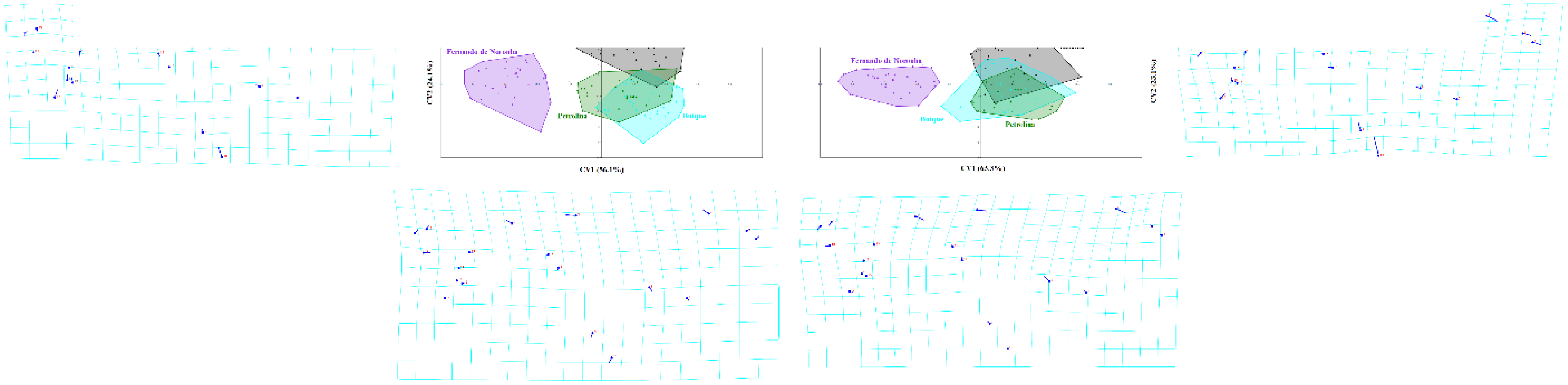


Figure 5. Canonical Variate Analysis (CVA) for wing shape of *Chrysomya megacephala* (female – left, and male – right) and the transformation grid of the wing shape of individuals in each axis. The vectors indicate the direction of the deformation direction.

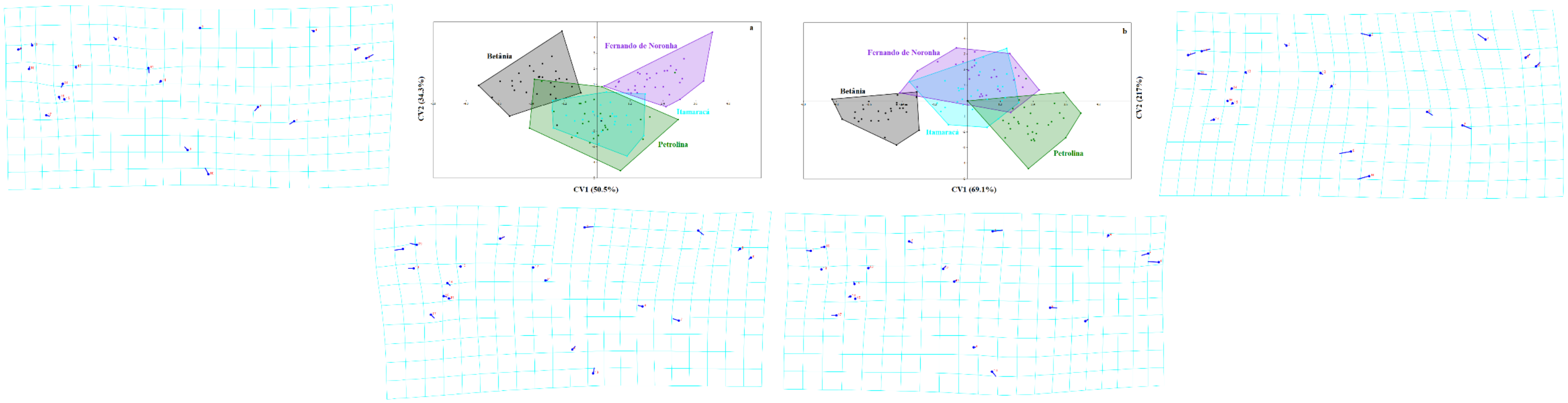


Figure 6. Results of cluster analysis (PLS) according correlation of the environmental factors and the wing shape of *Cochliomyia macellaria* along to eco-regional gradient. (left – female and right – male).

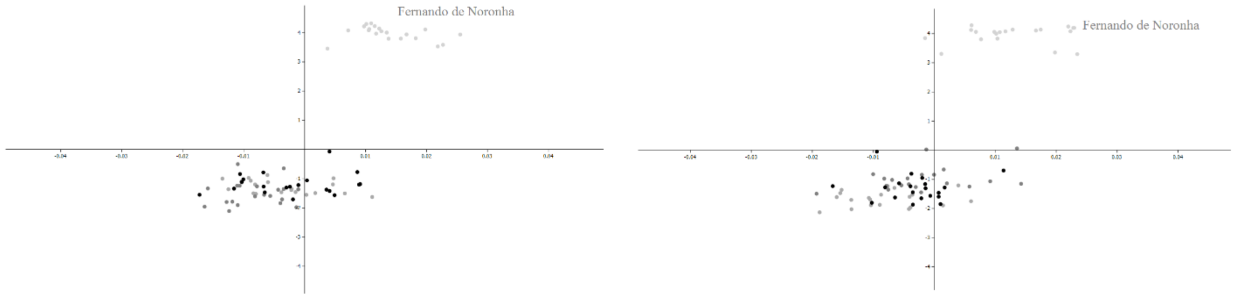
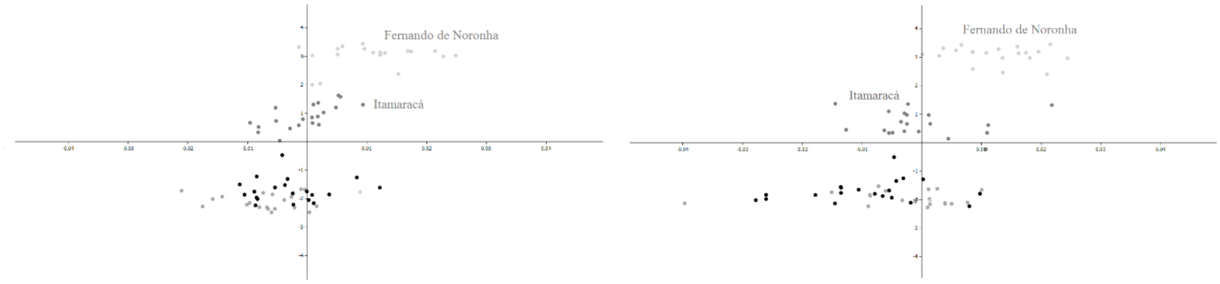


Figure 7. Results of cluster analysis (PLS) according correlation of the environmental factors and the wing shape of *Chrysomya megacephala* along to eco-regional gradient. (left – female and right – male).



CAPÍTULO 3

Esse capítulo segue as normas para publicação da Ecological Entomology

Running title: Effect of larval competition on blow fly species

Impact of competition with invasive *Chrysomya rufifacies* on life-history traits of native *Cochliomyia macellaria* (Diptera: Calliphoridae)

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Abstract. 1. The necrobiome is a unique microcosm, in which a range of organisms interacts in association with an ephemeral resource, such as vertebrate carrion. In many cases, arthropod communities associated with these resources can be restricted to a few dipteran species where competition is an important factor influencing their community structure.

2. Blow fly species frequently show different competitive abilities which, when associated with other types of behavior, such as predation or cannibalism, influence coexistence. Laboratory experiments were used to evaluate the effect of interspecific competition on bionomics and survivorship parameters in populations of native and invasive blow fly species at different larval proportions.

3. *Cochliomyia macellaria*, a native species, responded to the presence of *Chrysomya rufifacies*, an invasive species, with the sub-lethal effects decreasing concurrently with reductions in larval density of *C. rufifacies*. The larval characters most impacted by competition were length and weight with all *C. macellaria* dying when exposed to high proportions of *C. rufifacies*. Furthermore, increased densities of *C. rufifacies* impacted pupal development of *C. macellaria*, and resulting adults were smaller and had narrower wings.

4. For *C. rufifacies,* intraspecific competition had the greatest impact on larval survival.

5. Theses results lead us to conclude the pressure of competition in the larval phase – as a species-dependent behaviour – acts a main factor on development and bionomic parameters for both species.

Keywords: sub-lethal parameters, larval density, survivorship rate, insect development, consumptive effect, succession

Introduction

An animal carcass is a unique and ephemeral microcosm in which many organisms (i.e., vertebrate scavengers, insects and microbes) compete for resources (Weatherbee *et al*., 2017). Benbow *et al.* (2013) describe this microcosm as the necrobiome and highlights that, despite its ephemeral nature, complex interactions in food webs of associated ecosystems take place. The necrobiome of vertebrate carrion can be small and distinct; and communities developing within them have at their disposal a limited amount of energy, which is gradually used up by the activities of each member (Beaver, 1977). Species of Diptera play a key role in carrion recycling, using it as food and as a site for copulation and oviposition (Beaver, 1977). Frequently, the number of eggs or larvae deposited on a carrion by necrophagous flies exceeds its capacity (Kneidel, 1984). Because of that, competition – both intra and interspecific - strongly influences the structure of necrophagous dipteran communities.

Blow flies (Diptera: Calliphoridae) differ in their competitive abilities, which interfere with their coexistence by maintaining different species in patches in spite of their population sizes or by excluding one of them (Ullyett, 1950; Goodbrod & Goff, 1990). In the past few decades, four species in the Old World genus *Chrysomya* were introduced into the Americas. The first of these to become established in the continental USA was *Chrysomya rufifacies* (Macquart 1843), and since then populations of the native blow fly, *Cochliomyia macellaria* (Fabricius 1775), have been negatively affected (Wells & Greenberg, 1994; Byrd & Butler, 1996).

Dominance of invasive species of the genus *Chrysomya* over native ones have been reported in dead animals (Vasconcelos *et al*., 2016) and on human cadavers (Oliveira &

Vasconcelos, 2010), which stimulate empirical studies on the short- and long term effects of competition on insect assemblages. Quantitative data is applicable not only for the prediction of the invasive potential of exotic species but also for the practical use in forensic investigations, because larval age - the main predictor of the post-mortem interval - is thought to depend on resource competition. So far, laboratory studies between native and invasive blow fly species have demonstrated that the effect of larval competition on larval survivorship or development time depends not only on the density of the larval masses but also on the time of resource colonization (So & Dudgeon, 1989; Wells & Greenberg, 1992; Brundage *et al*., 2014). However, the complexity of interactions calls for an integrated assessment of morphological and bionomical parameters of both larval and adults exposed to competition.

In this scenario, we investigated the effect of *C. rufifacies* on, (a) larval development – total larval length, weight and development time to pupa; (b) dry weight of adults; (c) wing morphology of the adults; (d) overall survivorship. According to these objectives, we tested the following hypotheses: i) there is a negative correlation between the larval proportion of *C. rufifacies* and larval parameters of *C. macellaria*; ii) due to the predatory behaviour of *C. rufifacies*, intraspecific competition promotes a significant decrease in the larval and adults' life-history; and iii) interspecific competition reflect in the deformed wings of adults of *C. macellaria*.

Materials and Methods

Blowflies colonies

Larvae of both species used in this study were obtained from colonies at the Forensic Laboratory for Investigative Sciences (FLIES), Texas A&M University, USA, established in 2009, which was genetically enhanced by introducing adults sampled on campus in 2016. Adults were maintained in 30 x 30 x 30 cm cages (BioQuip®, Rancho Dominguez, CA, USA) placed in a walk-in growth chamber at 27.0 ± 1.0 °C, 60.0% RH, and LD 12:12 h photoperiod. Granulated sugar, buttermilk powder and water were provided *ad libitum*, and 50 g of bovine liver were offered to induce oviposition. Eggs were placed in separate plastic cups containing 50 g of fresh liver. Each cup was placed inside a 1 L mason jar containing 50% of the volume with sterilized sand. After hatching, larvae were fed 100 g of fresh liver until pupation.

Experimental design: Effect of competition on life history traits

Effects of intra- and interspecific competition were assessed by measuring bionomical parameters in larval populations of *C. macellaria* and *C. rufifacies* exposed at different proportions. The experimental arena consisted of a 710 mL transparent plastic container (15 x 11 x 8 cm) with 180 mL of sterilized sand, plus 100 g of fresh beef liver. We designed six treatments divided into pure and mixed colonies. The mixed colonies presented *high* or *moderate* densities of the invasive or native species (Table 1). Larvae in containers were maintained in the incubator previously described until adult emergence. Throughout the experiments, the position of the containers was randomized in the incubator after each observation.

We analysed the life-history traits of insects at two developmental stages: i) *larvae:* growth (length in mm), weight gain (g) and development time (h), and ii) *adults*: overall survivorship rate (%), wing size and shape of emerged individuals (morphometric geometric analysis, see below) and dry weight (g). Larval samples were taken from each replicate of each treatment every 8 h. During each observation, a number of larvae corresponding to 20% of each treatment were removed and larval length, weight, and instar were recorded. Larvae were cooled (-20 °C) for five seconds prior to each measurement, and returned to the appropriate container after being measured. The procedure was repeated until pupation. Each pupa was removed and transferred to a separate container (50 mL) and placed in the incubator previously described. Similarly to larval observations, pupae were monitored every 8 h until the emergence of adults. Surviving adults were sacrificed 6 h after emergency, stored in ethanol 95% for three days before being dried in an oven (50°C for 48 h) after which the dry weight recorded. For this study, five replicates were performed, from five generations of both species.

Effect of larval competition on wing shape and size of adult flies

We used Morphometric Geometric Analysis to evaluate the size and shape of wings of adults from all treatments, as wing size tends to reflect the size of the adult body among blowflies (Dujardin, 2008). To standardize the samples, we only used right wings of males of both species. All wings were photographed with a Lumenera camera coupled to a stereomicroscope (Meiji, model EMZ-8TR) using Infinity® software. Nineteen wing type I landmarks were digitized for both species, which included 15 landmarks proposed by Lyra *et al.*

(2010) in addiction to four new landmarks (Fig. 1). We used a subsample of 20% of each species in each treatment for evaluation.

Data Analysis

We used Analysis of Variance (ANOVA) to test the effect of competition in the different treatments and larval densities on dry weight of adult flies, followed by post-hoc test of LSD ($P < 0.05$), both in single as mixed colonies. The Pearson correlation was carried out to evaluate the life-history traits of insects between the immature and adult stages, taking in consideration the variables for each stags previously described.

To test for differences in average size, we compared wing size among the treatments using an ANOVA, considering the centroid size (CS). CS is defined as the square root of the sum of squared distance of landmarks from the center of gravity of a configuration (Bookstein, 1991). We tested the repeatability of coordinates (x, y) by Multivariate Analysis of Variance (MANOVA) with 10 males selected randomly, photographed twice by the same observer. MANOVA and pairwise tests were carried out on the landmark data to compare wing shape among treatments. The populations were ordered in a reduced space using canonical variable analysis (CVA) by MorphoJ® software. CVA is a technique that maximizes the separation of populations because each axis is constrained to represent the maximum between-group variance. The statistical analysis was carried out in Statistica®, MorphoJ®, and Past® software ($\alpha < 0.05$).

Results

Effect of competition on larval parameters

All life-history traits of *C. macellaria* were affected by the presence of the invasive species. A significant difference among the treatments was evidenced for both weight ($F_{9;40}$ = 9.031; $P < 0.001$) and length ($F_{9;40}$ = 5.776; $P < 0.001$). Specifically, larvae from the pure colonies of *C. macellaria* were heavier than those in mixed colonies, with significant differences from all other larval proportions in mixed colonies (Table 2). Mean weight of *C. macellaria* was inversely proportional to larval density of invasive species (Fig. 2a). No statistical difference in the weight of 1st ($F_{9;40}$ = 1.730; P = 0.113) or 2nd ($F_{9;40}$ = 1.098; P = 0.385) instar *C. macellaria* from pure or mixed colonies was observed. Interestingly, 3rd instar *C. macellaria* larvae in the presence of the low *C. rufifacies* density were heavier than those in the control (Fig. 2d).

Competition influenced larval length of *C. macellaria*, with the mean length being inversely proportional to the density of invasive species (Fig. 3a). Only larvae from the HPN treatment were similar in length to those in the pure colony, with statistical difference among all the mixed and pure colonies (Table 2). Furthermore, a significant ($P < 0.05$) difference in the length of *C. macellaria* between HPI and MPI was observed, which were smaller under high densities of invasive species. We did not detect any difference in the overall larval length across the treatments (Fig. 3b, c, d). The transversal analysis between the pure colonies of the two species showed similarity in both larval parameters (Table 2). In contrast, mixed colonies under high and moderate proportion of invasive species, and MPN presented an increase in the larval total length of *C. macellaria* in the opposite direction of *C. rufifacies* larval density. Neither weight or larval length differred for *C. rufifacies*, even when analysed by instar (Fig. 2 and 3).

Chrysomya rufifacies showed a slight positive correlation between overall larval weight and length (r = 0.569; $P < 0.01$ – Fig. 4a), also in the 2nd and 3rd instars (Fig. 4e, f). Larvae gained more weight in the 2nd instar, which is reflected in the total larvae size as well (r = 0.713; $P < 0.001$). For *C. macellaria*, a strong correlation was evident when the overall larval weight and larval length were analysed (r = 0.816; $P < 0.001$ – Fig. 5a). In contrast to the invasive species, *C. macellaria* gained more weight in the 3rd instar, and this increase is correlated with the total larvae size as well (3rd instar r = 0.749; $P < 0.001$ – Fig. 5d, e, f).

Effect of competition on development larval time

Competition in pure and mixed colonies did not affect larval development time for *C. rufifacies,* which spent an average of 46.4 ± 10.4 h developing from 1st instar to the pupal stage across treatments (Fig. 6). Development time was similar for all treatments when each instar was analyzed (12.8 ± 4.4 h in 2nd instar, and 25.6 ± 6.7 h in 3rd instar). On the other hand, the interspecific competition negatively affected larval development time of *C. macellaria*, which spent less time until pupation when compared with the pure colony (Fig. 6). Even in the low proportion *C. rufifacies* treatment, the time needed for *C. macellaria* to reach the pupal stage was 37% faster than in pure colonies. In treatments under high and moderate proportions of *C. rufifacies*, all 2nd instar *C. macellaria* larvae were predated in less than 10 h. There was no difference in development time in 2nd instar *C. macellaria* larvae across treatments (Fig. 7).

However, when the 3^{rd} instar was analysed, larvae from the pure colony needed almost 45% more time to pupate than the treatment under low proportion of invasive species.

Effect of competition on adult dry weight and survivorship rate

Competition affected the mean dry weight of *C. macellaria* ($F_{9;40} = 11.426$; $P < 0.001$) in an inversely direction to density of *C. rufifacies*. For *C. rufifacies* neither intra or interspecific competition affected the overall mean dry weight. The treatment HPI had no *C. macellaria* survivor adults, and therefore could not be used for this analysis. *Cochliomyia macellaria* from pure colonies were heavier than those resulting from mixed colonies. Transversal test between the pure colonies did not show differences in the dry weight for both species ($P = 0.788$). However, in all mixed cultures *C. rufifacies* were significantly heavier than the native species ($P < 0.05$).

The survivorship rate was different according to the treatment for both species. Survivorship of *C. macellaria* was higher in pure colonies (81.4% ± 11.8%) and decreased significantly (17.1% ± 9.4 to 0) with increasing densities of *C. rufifacies*, with the lowest values in the treatment under high presence of invasive species (Table 3). For *C. macellaria*, only those from the control differed statistically from the mixed colonies ($F_{4:20} = 27.531$; $P < 0.001$). *Chrysomya rufifacies* exhibited greater (69.3% ± 24.5%) survivorship rate when exposed to low proportion of competitors (Fig. 8). Although there was no statistical difference among the treatments ($F_{4:20} = 1.328$; $P = 0.294$), mixed cultures with MPI and MPN presented higher survivorship rate (69.3% ± 24.5% and 69.0% ± 35.8%, respectively) of *C. rufifacies*. In pure colonies, *C. rufifacies* showed the smallest values of survivorship. Larval weight of *C. rufifacies* did not correlate with adult dry weight (Fig. 4b); however, a positive correlation between the total larval length and dry weight of adults was evidenced (Fig. 4c). On the other hand, for *C. macellaria,* the biological parameters from larvae displayed a strong positive correlation with adult dry weight (r = 0.703; $P < 0.001$ – larval weight gain, and total larval length r = 0.674; $P < 0.001$ – Fig. 5b, c).

Effect of competition on wing size and shape of adult flies

Comparison of data across trials demonstrated good repeatability for *x*, *y* coordinates, ranging from R = 0.88 to R = 1.00 (R ≈ 0.96), even for centroid size (R ≈ 0.99). Competition impacted wing size differently for each species. For *C. macellaria,* the treatments HPI and MPI were not analysed, because data were insufficient (low survivorship when exposed to *C. rufifacies*). Despite this, the pattern of smaller individuals of native species being inversely proportional to the density of *C. rufifacies* was maintained. The MPN treatment showed the lowest values of CS for *C. macellaria*, differing from the others ($F_{2;27} = 9.918$; $P < 0.001$). Low presence of *C. rufifacies* in mixed cultures did not affect the wing size of native species; in fact, they were similar to those resulting from the pure culture ($P = 0.919$). Analysis of Variance showed an irregular variation of wing size for *C. rufifacies* across treatments (Fig. 9). The pure and mixed colonies with moderate proportions of *C. rufifacies* produces adults with the smallest wings. In contrast, adults from the HPN exhibited larger wings, being different from the others, except from the mixed colony with high proportion of invasive species (Table 4).

Contrarily to size, wing shape did not differ among treatments for *C. macellaria* (Wilks´s $\lambda = 0.00022$; d.f. = 27;54; $P = 0.334$). The ordination of populations (each population means a different treatment) by CVA showed that the first axis explained 64.4% of variations in the wing shape for this species. This axis is responsible to segregate the mixed colonies with MPN and HPN (Fig. 10a). The segregation of the pure and mixed colonies is clear in the second axis. Despite this segregation, the cross-validation test by a Discriminant Analysis – with 1,000 permutations – showed a significant difference only between the pure colony and the treatment under moderate proportion of native species (Table 5). The wings from the treatment under moderate proportion of native species displaced the landmarks 9, 10 and 18 to the centre of wing. Because of that, flies from this treatment exhibited a design of wings slightly narrower than the others (Fig. 10a).

For *C. rufifacies*, MANOVA results showed a significant difference in the shape wings among the treatments (Wilks´s $\lambda = 0.00124$; d.f. = 34;136; $P < 0.05$). The ordination of populations by CVA showed that the first and second canonical variable jointly explained approximately 85% of all variation in the wing shape. The axis 1 displayed a segregation of control treatments from the others (Fig. 10b). The presence of control population in the negative values of the CVA illustrates a pattern of wings narrower than other treatments, with landmarks 2, 9, 10 and 17 displaced in direction to the wing center (Fig. 10b). Wings of flies from the control were narrower than those from other treatments, which was corroborated by Discriminant Analysis, with significant difference between control and MPI and HPN (Table 6).

Discussion

Effect of competition on larvae in pure and mixed colonies

Differential responses by competing species were determined to depend on the stage as well as on the identity of competitors (i.e., intra- vs interspecific), as evidenced by the absence of variability in the larval parameters of *C. rufifacies* among treatments. However, this absence of variability may mask potential biological effects on the species. So and Dudgeon (1989) described two types of response to intraspecific competition among dipteran larvae: 1) positive correlation between mortality and larval density; and 2) negative correlation between adult morphology (i.e., size and weight) and larval density. In our study, *C. rufifacies* showed the first scenario, with a survivorship rate in pure cultures almost 50% lower than in mixed cultures. One possible explanation is that the 1st instar of the invasive species is entirely necrophagous, but under crowded or starved conditions, the 2nd and 3rd instars can develop predatory or cannibalistic behaviour (Wells & Greenberg, 1992; Baumgartner, 1993).

Chrysomya rufifacies is considered a primary colonizer of carrion (Baumgartner, 1993) and larvae can resort to predation or cannibalism under limited resources (Shiao and Yeh, 2008) allowing to survive even as a secondary colonizer. However, Brundage *et al*. (2014) demonstrated that *C. rufifacies* had higher fitness when arrived at a resource concurrently with *C. macellaria*. It appears that *C. rufifacies* is not delaying colonization to avoid competition, suggesting that predator-pray relationship between *C. rufifacies* and *C. macellaria* is more important than competition for food (Brundage *et al.* 2014).

The greatest survival rate of *C. macellaria* in single colony (c.a. 81%) may be due to several innate mechanisms, such as the larval aggregation in a single mass burrowing on the food medium for continuous feeding. The movement of numerous mouth-hooks combined with secretions of salivary proteolytic enzymes increase the efficiency of the feeding process (Kitching, 1976; Goodbrod & Goff, 1990).

Cochliomyia macellaria exhibits a density dependent sensitivity to the presence of a competitor with manifestation of this impact from the minimum threshold. In our study, the sub-lethal effects were directly proportional to the density of larvae of the invasive species. The presence of *C. rufifacies* impacted the fitness of *C. macellaria*, which took 43 to 63% less time to pupate in mixed cultures when compared to the control. This correlation indicates that larval density is a crucial and species-dependent factor in interspecific competition and that larval development time is a secondary factor affected by the competition.

Shiao and Yeh (2008) argued that early contact of *C. rufifacies* with larvae masses of *Chrysomya megacephala* (Fabricius, 1974) is probably only to facilitate their feeding process and larval development, instead of predation or cannibalism, since the predatory behaviour of this species occurs for those in 2nd and 3rd instar (Goodbrod & Goff, 1990). This idea is corroborated by Brundage *et al*. (2014), where *C. rufifacies* required resource modification by a primary colonizer to allow efficient nutrient intake.

If *C. rufifacies* is a primary or secondary carrion fly, and if such an ecological role acts as a trigger to initiate its predatory behaviour is still debatable; however, when it occurs, *C. macellaria* can avoid competition with the invasive species in some instances. One hypothesis could be that, by preceding *C. rufifacies* in succession, *C. macellaria* is able to survive (Wells & Greenberg, 1994) as it develops faster than later arriving *C. rufifacies* (Boatright & Tomberlin, 2010; Flores *et al*., 2014). Simultaneous arrival, such as in our experiment as well as the Brundage *et al.* (2014) study, resulted in decreased *C. macellaria* survival. These results agree with the second proposed hypothesis of So & Dudgeon (1989) for interspecific competition.

Newly hatched *C. rufifacies* initially aggregate when in pure cultures (Shiao & Yeh, 2008 and personal observation). If other species, such as *C. megacephala* (Shiao & Yeh, 2008) or *C. macellaria* (present study), arrive and colonize the substrate, *C. rufifacies* larvae tend to invade the other species' masses and force the competitor to disperse from the food earlier. The same observation was made by Brundage *et al*. (2014), with *C. rufifacies* forcing *C. macellaria* to leave the resource before reaching the minimum viable weight necessary for successful pupation.

Effect of larval competition in adult parameters

In the ephemeral microcosm represented by carrion, high levels of larval competition among necrophagous blowflies occur, and one consequence of this antagonism is the generation of small adult individuals (So and Dudgeon, 1989; Von Zuben & Godoy, 2000). Several studies describe a direct relationship between the quantity and quality of alimentary resource acquired by larvae with the size and weight of adult (Sullivan & Sokal, 1963; Williams & Richardson, 1983; Goodbord & Goff, 1990; Reis *et al*., 1994). Similarly, we observed that while the dry weight of adult *C. rufifacies* did not differ among the treatments, wings of *C. rufifacies* were smaller and narrower under high competition when compared with flies exposed to low competition. These results indicate that the pressure suffered by larvae could have interfered with the acquisition of necessary food resources for normal adult formation. Accordingly, for *C. macellaria*, both dry weight adults and wing size were negatively impacted as consequence of larval competition. Nicholson (1957) noted oscillations in adult population size with high densities of adults leading to high density of larvae, which could not be supported by the constant supply of resource yielding smaller larvae and fewer adults. Furthermore, Brundage *et al*. (2014) observed *C. macellaria* larvae arriving within two days after *C. rufifacies* exhibited the most dramatic decrease in survivorship, pupal weight, and adult longevity of all treatments.

In experiments using different larval densities of necrophagous dipterans, Von Zuben *et al.* (2010) noted significant variation in adult weight among treatments. They suggest several scenarios exist with the same level of aggregation, but with larval densities and quantity of source proportionally different. For example, the results from Von Zuben *et al.* (2010) showed have variation on weight with the increase of larval proportion per gram of food. Furthermore, even considering the same larval proportion per gram of food in each larval density the results of weight were significantly different, did not producing necessary adults with the same weight and size. In this study, interspecific larval competition did not affect the adult parameters for *C. rufifacies*, and only a weak correlation between larval length and dry weight of adult were found In contrast, *C. macellaria* responded to interspecific larval competition, as a consequence of result is the larval feeding behaviour. The presence of a potential competitor accelerated feeding process and consequently incomplete nutrient absorption, resulting in smaller and lighter adults. Increasing intraspecific competition may lead to decreased development times at several life stages, but was also determined to affect the size and weight of the resulting larvae/pupae produced (Ullyett, 1950; Goodbrod & Goff, 1990).

Although the relationship between larval competition and adult size is relatively known, alterations in wing shape as a consequence of intraspecific competition, have not been, to our knowledge, investigated for the species in question. Only *C. macellaria* showed a significant difference in wing shape as a consequence of larval competition. This absence of allometric factor indicates that the presence of *C. rufifacies* in mixed cultures forces the early abandonment of the resource by *C. macellaria* which, in turn, results in early metamorphosis associated with different wing pattern (e,g,, narrower wings). Food shortage leads to morphological flight apparatus change in holometabolous insects.. Because small and narrow wings have an inverse relationship to flight ability (Dudley, 2000), our results suggest that a reduced flight performance may be in indirect disadvantage faced by surviving *C. macellaria* adults when exposed to an invasive competitor at larval stage.

Bionomical and behavioral data obtained in experiments on larval competition under controlled conditions in the laboratory can only be validated by their extrapolation to field situations. Our experimental model derives from field observations of coexistence of *C. rufifacies* and *C. macellaria* in several scenarios using animal carcasses and human cadavers. For example, *C. rufifacies* dominated the assemblages of necrophagous insects on bear carcasses in Florida, where they represented over 85% of all individuals (compared to only 3% of *C. macellaria*) (Swiger *et al*., 2014). In Colombia, *C. rufifacies* was also the most abundant species on human cadavers (Barreto *et al*., 2002), which reinforces its competitive potential. Expansion of the geographical distribution of *C. rufifacies* towards South America may lead to its widespread establishment, and molecular techniques have been used to determine if it has reached other countries, such as Brazil (Grella *et al*., 2015). The ecological consequences of this imminent arrival can only be (partially) predicted by quantitative studies. Data presented here highlights the risks of survivor to native species in a small-scale. Considering the enormous biotic potential, fecundity and behavioral plasticity of the species of the *Chrysomya* complex, it is likely that the colonization of ephemeral resources may be dominated by invasive species, unless native blowflies are demonstrated to exploit a wider range of substrates. Under a practical standpoint, comparative data on the bionomics of cadaver-dwelling larvae can validate the estimation of the post-mortem interval, the most useful contribution of forensic entomology. Lastly, our results shed some light to the applicability of morphometric tools to better understand the consequences of the complex mechanism of competition on decomposition process by necrophagous flies. The next step should prioritize wing morphometric as a complementary tool to analyse the flight and dispersal ability of the adults.

Acknowledgments

We thank the following people and institutions: Fundação de Amparo a Ciência e Tecnologia de Pernambuco (FACEPE), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) Brazil for the financial support (international scholarship); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Ashleigh Faris and Adrianna Tirloni for the invaluable help in maintenance of the colonies; Rodrigo Correa and Heo Chong Chin for the advice on the experimental design.

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TABLES:

Table 1. Categorical description of the treatments according to larval proportion, including the control for both invasive (*Chrysomya rufifacies*) and native (*Cochliomyia macellaria*) species.

Treatment	Category	Abbreviation	Proportion of larvae
Control of invasive fly	Pure invasive colony	PIC	100 invasive
Treatment 2	High proportion of invasive species	HPI	80 invasive / 20 natives
Treatment 3	Moderate proportion of invasive species	MPI	60 invasive / 40 natives
Treatment 4	Moderate proportion of native species	MPN	40 invasive / 60 natives
Treatment 5	High proportion of native species	HPN	20 invasive / 80 natives
Control of native fly	Pure native colony	PNC	100 natives

Table 2. Result of post-hoc ANOVA test *LSD* for *Chrysomya rufifacies* and *Cochliomyia macellaria* among the treatments. Significant values for both variables Weight (above diagonal) and Length (below diagonal) are in bold.

	Weight	*C. rufifacies*					*C. macellaria*				
Length		PIC	HPI	MPI	MPN	HPN	PNC	HPI	MPI	MPN	HPN
C. rufifacies	PIC	-	0.67	0.70	0.77	0.57	0.33	< **0.01**	< **0.01**	< **0.01**	0.06
	HPI	0.86	-	0.96	0.47	0.89	0.59	< **0.01**	< **0.01**	< **0.01**	< **0.05**
	MPI	0.64	0.76	-	0.50	0.85	0.56	< **0.01**	< **0.01**	< **0.01**	< **0.05**
	MPN	0.81	0.95	0.81	-	0.39	0.21	< **0.01**	< **0.01**	< **0.01**	0.10
	HPN	0.74	0.87	0.89	0.92	-	0.69	< **0.01**	< **0.01**	< **0.01**	< **0.05**
C. macellaria	PNC	0.26	0.33	0.50	0.37	0.42	-	< **0.01**	< **0.01**	< **0.01**	< **0.01**
	HPI	< **0.01**	< **0.01**	< **0.01**	< **0.01**	< **0.01**	< **0.01**	**-**	0.99	0.16	< **0.05**
	MPI	< **0.01**	< **0.01**	< **0.01**	< **0.01**	< **0.01**	< **0.01**	0.48	-	0.17	< **0.05**
	MPN	0.06	< **0.05**	< **0.05**	< **0.05**	< **0.05**	< **0.01**	0.06	0.22	-	0.28
	HPN	0.78	0.65	0.45	0.60	0.54	0.16	< **0.01**	< **0.01**	0.11	-

Table 3. Average survivorship rate (%) for *Chrysomya rufifacies* and *Cochliomyia macellaria* with respective standard deviation for five replicates.

Treatments	***Chrysomya rufifacies***	***Cochliomyia macellaria***
PIC	36.2 ± 21.7	-
HPI	59.5 ± 25.1	0
MPI	69.3 ± 24.5	2.5 ± 5.5
MPN	69.0 ± 35.8	16.3 ± 27.3
HPN	59.0 ± 20.7	17.1 ± 9.4
PNC	-	81.4 ± 11.9

Table 4. Result of post-hoc ANOVA test *LSD* for *C. macellaria* (above) and *C. rufifacies* (below) according to Centroid Size. Significant values are in bold.

Treatments	Control	T2	T3	T4	T5
Control	-	**0.0001**	**0.0001**	**0.0004**	0.9865
HPI	**0.0001**	-	1.0000	**0.0001**	**0.0001**
MPI	0.2321	**0.0001**	-	**0.0001**	**0.0001**
MPN	**0.0350**	0.0782	**0.0002**	-	**0.0002**
HPN	**0.0001**	0.2155	**0.0001**	**0.0003**	-

Table 5. Results of Discriminant Analysis for wing shape of *Cochliomyia macellaria* among the treatments. The p-value is resultant from the cross-validation test (1000 permutations).

Discriminant Analysis	**Control - MPN**	**Control - HPN**	**MPN- HPN**
Mahalanobis Distance	4.464	2.228	3.401
P	< 0.001	0.471	0.076

Table 6. Results of Discriminant Analysis for wing shape of *Chrysomya rufifacies* among the treatments. The p-value is resultant from the cross-validation test (1000 permutations).

Combination	**Mahalanobis Distance**	***P***
Control – HPI	3.409	0.076
Control – MPI	3.978	< 0.05
Control – MPN	3.118	0.599
Control – HPN	3.586	< 0.005
HPI – MPI	3.173	< 0.05
HPI – MPN	2.495	0.372
HPI – HPN	3.471	< 0.05
MPI – MPN	3.196	< 0.05
MPI – HPN	3.017	0.093
MPN – HPN	2.261	0.542

FIGURES:

Figure 1. Wing of *Chrysomya rufifacies* (left) and *Cochliomyia macellaria* (right) showing the 19 landmarks used in the morphometric analyses.

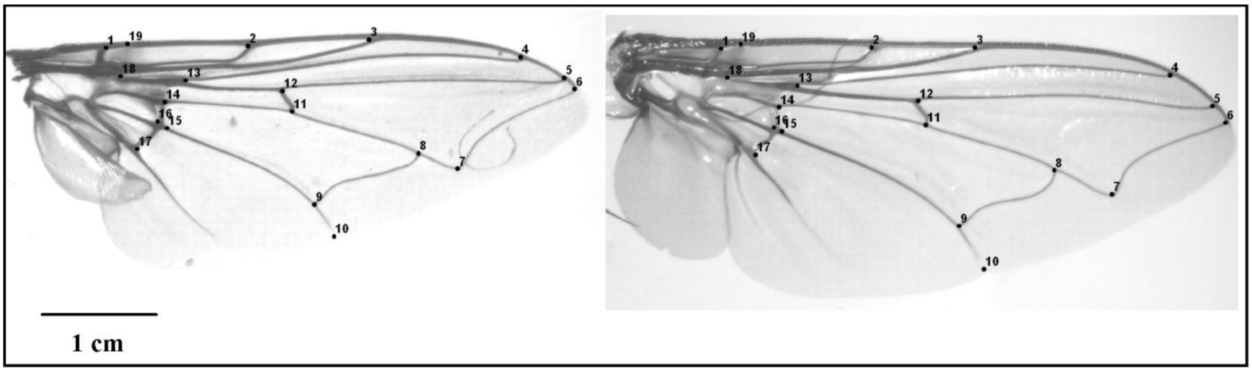


Figure 2. Mean weight ± SD for *Chrysomya rufifacies* (black) and *Cochliomyia macellaria* (grey): a) overall mean weight; b) 1st instar mean weight; c) 2nd instar mean weight and d) 3rd instar mean weight.

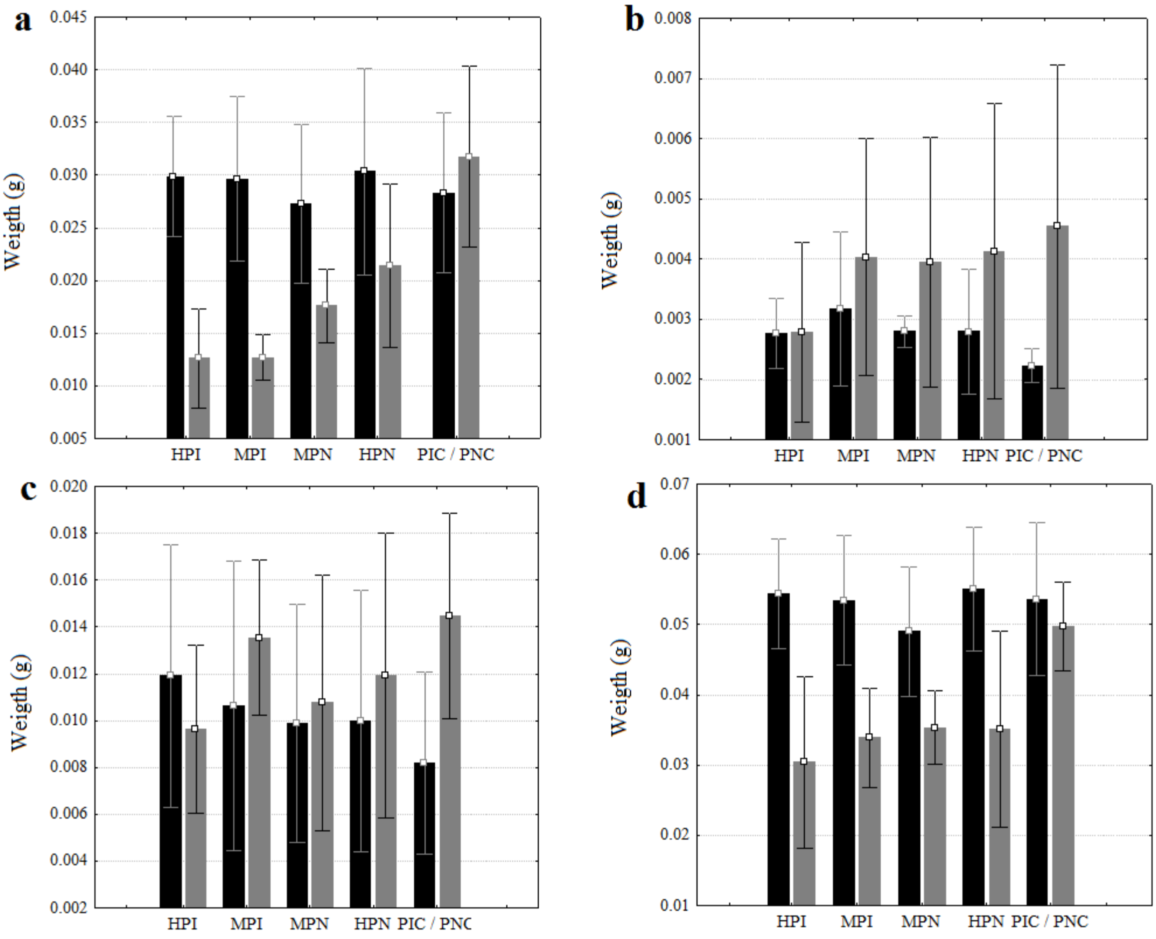


Figure 3. Mean length ± SD for *Chrysomya rufifacies* (black) and *Cochliomyia macellaria* (grey): a) overall mean length; b) 1st instar mean length; c) 2nd instar mean length and d) 3rd instar mean length.

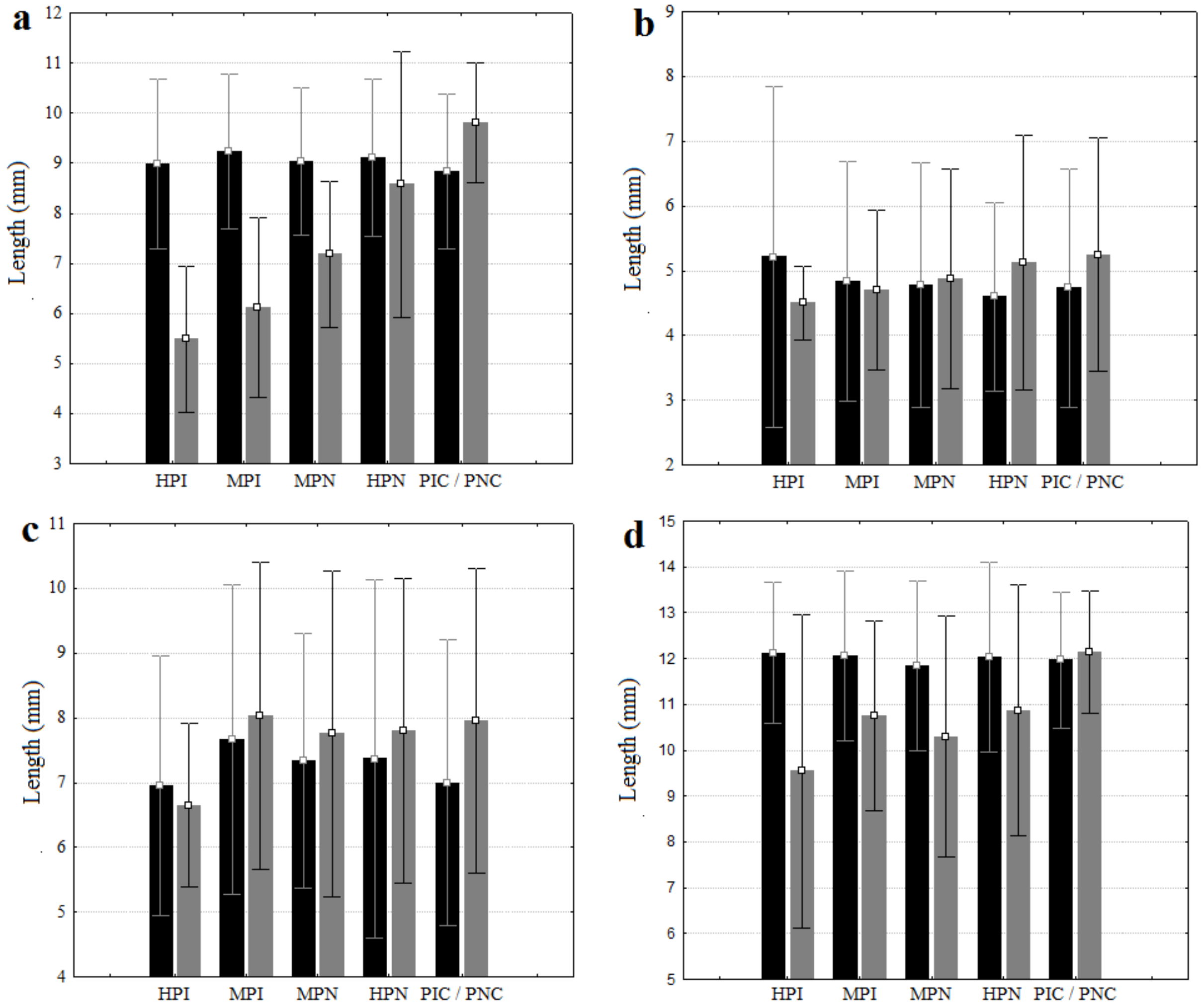


Figure 4. Analyses of Pearson’s correlation for larval and adult parameters of *Chrysomya rufifacies*: a) total larval length and weight; b) dry weight of adult flies and total larval length; c) dry weight of adult flies and total larval length; d) total larval length and weight for 1st instar; e) total larval length and weight for 2nd instar; and f) total larval length and weight for 3rd instar.

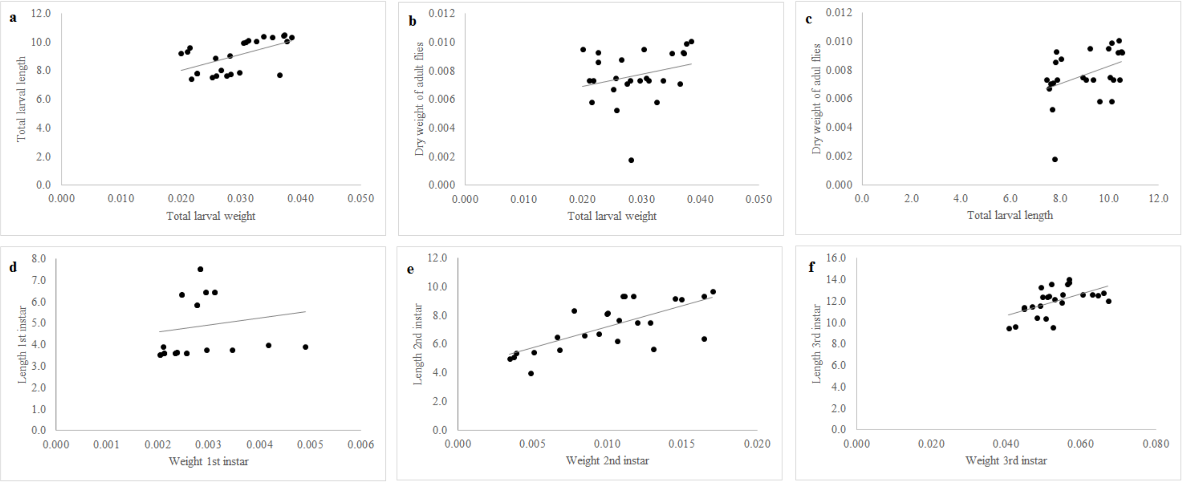


Figure 5. Analyses of Pearson’s correlation for larval and adult parameters of *Cochliomyia macellaria*: a) total larval length and weight; b) dry weight of adult flies and total larval length; c) dry weight of adult flies and total larval length; d) total larval length and weight for 1st instar; e) total larval length and weight for 2nd instar; and f) total larval length and weight for 3rd instar.

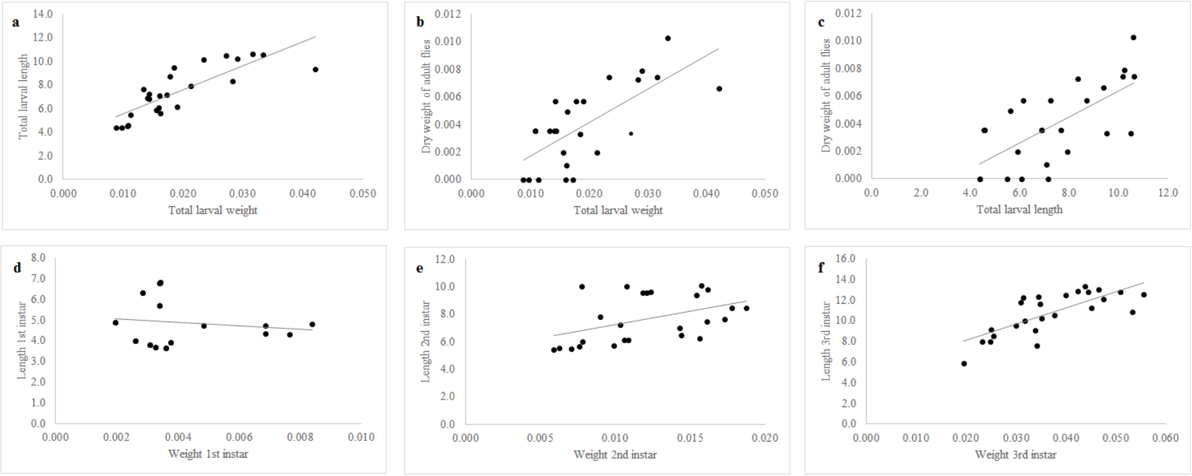


Figure 6. Development larval time for *Chrysomya rufifacies* (gray) and *Cochliomyia macellaria* (black).

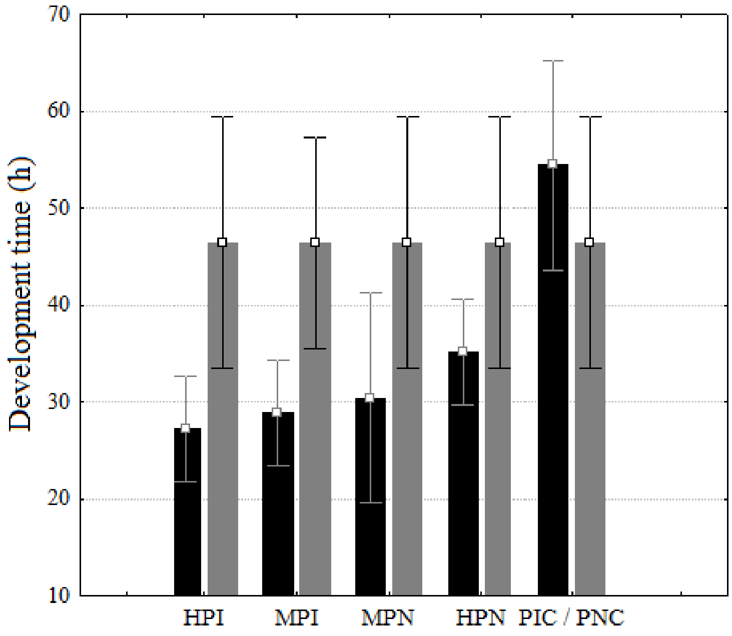


Figure 7. Development larval time for second (black) and third instar (gray) of *Cochliomyia macellaria*, with a Kruskal Wallis analyses for each instar.

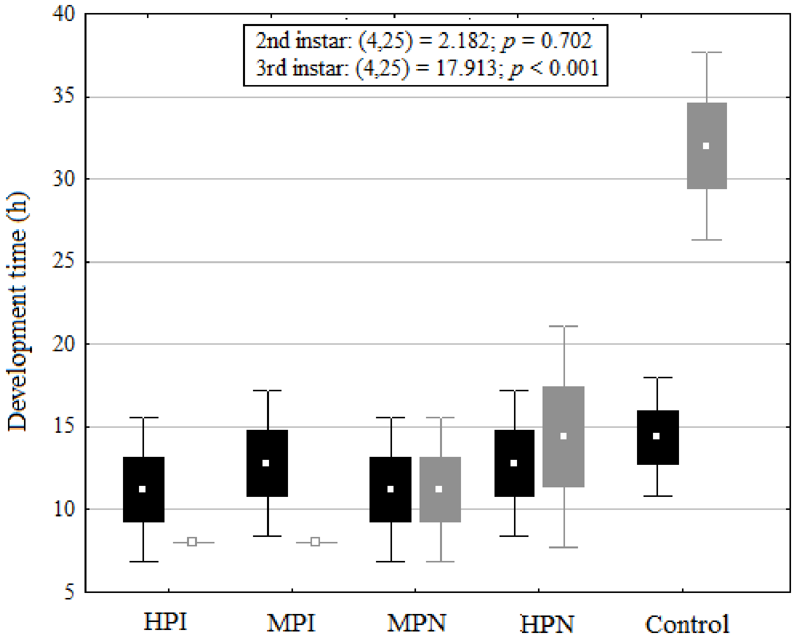


Figure 8. Survivor rate of *Chrysomya rufifacies* in pure (control) and mixed cultures.

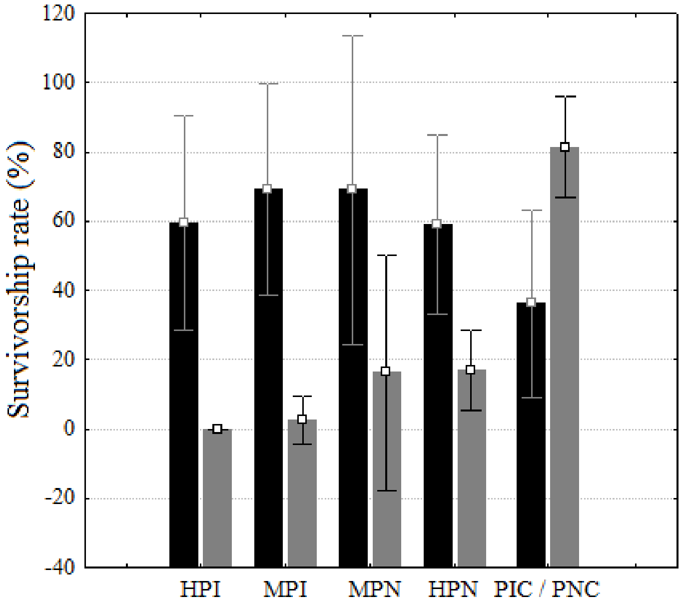


Figure 9. Wing size (mean of centroid size) for *Chrysomya rufifacies* across the treatments.

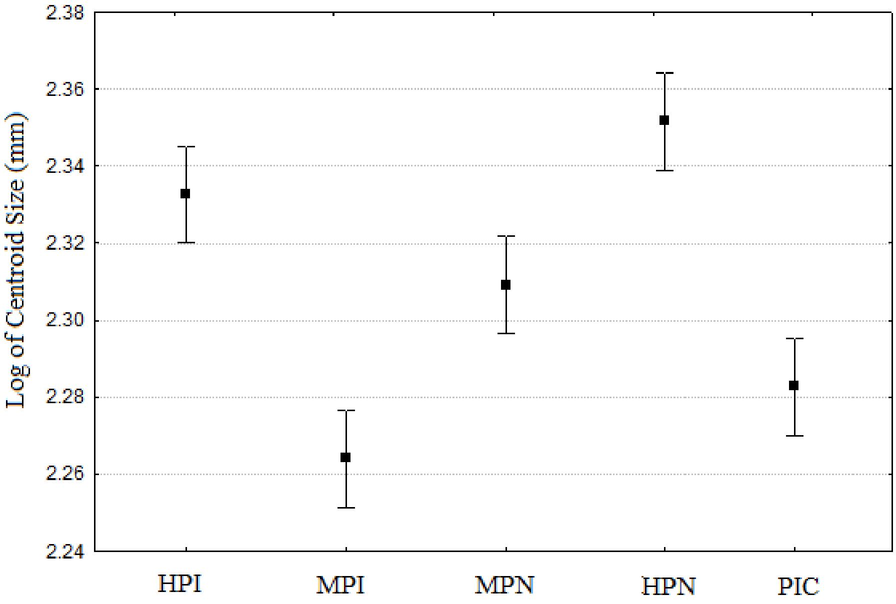
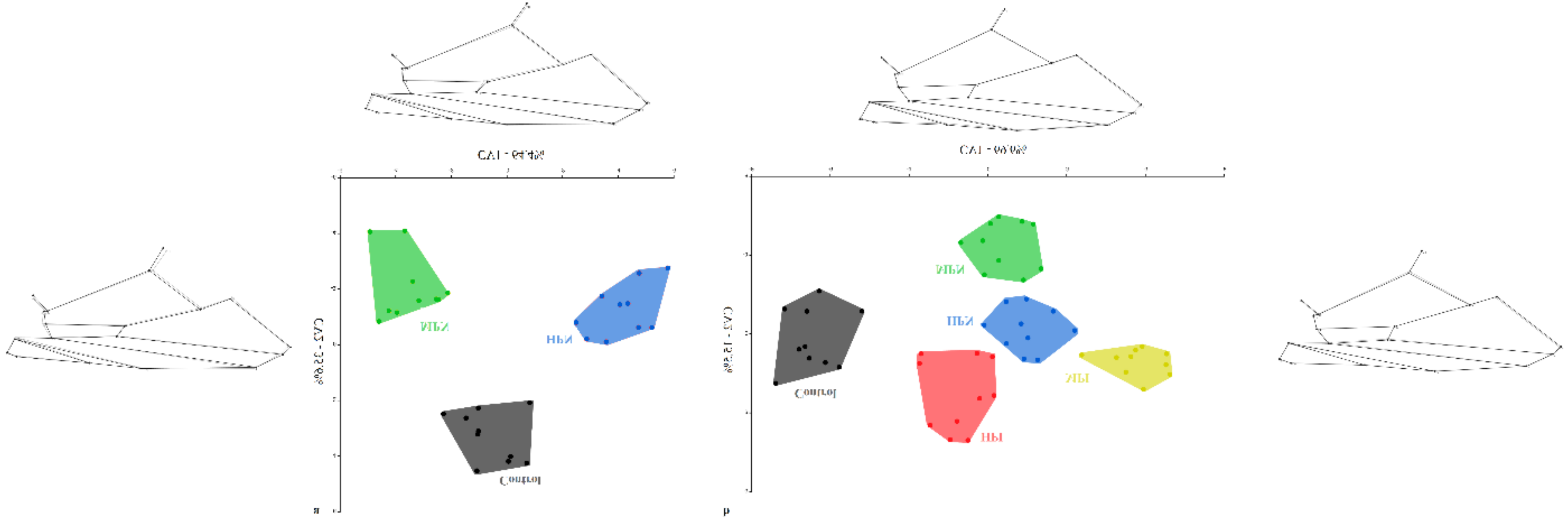


Figure 10. Canonical Variate Analysis (CVA) for wing shape of *Cochliomyia macellaria* (a) and *Chrysomya rufifacies* (b), with the graphic reconstruction of the wing shape of individuals in each axis. The lines in gray represent the average configuration of the wing, and those in black represent the canonical variable



Anexos

Licença de coleta

Cronograma de atividades

#	Descrição da atividade	Início (mês/ano)	Fim (mês/ano)
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#	Nome	Função	CPF	Doc. Identidade	Nacionalidade
1	Simão Dias de Vasconcelos Filho	Colaborador	547.389.464-04	2686772 SSP-PE	Brasileira

Locais onde as atividades de campo serão executadas

#	Município	UF	Descrição do local	Tipo
1		PE	PARQUE NACIONAL MARINHO DE FERNANDO DE NORONHA	UC Federal
2		PE	ÁREA DE PROTEÇÃO AMBIENTAL DE FERNANDO DE NORONHA	UC Federal

Atividades X Táxons

#	Atividade	Táxons

1	Coleta/transporte de espécimes da fauna silvestre in situ	Insecta (*Qtde: 150)

* Qtde. de indivíduos por espécie/localidade/unidade de conservação, a serem coletados durante um ano.

Material e métodos

1	Método de captura/coleta (Invertebrados Terrestres)	Outros métodos de captura/coleta(Para a captura dos insetos serÃ£o utilizadas armadilhas do modelo de Ferreira (1978) com isca)

Destino do material biológico coletado

#	Nome local destino	Tipo Destino
1	UFPE - UNIVERSIDADE FEDERAL DE PERNAMBUCO	coleção

Nome: Rodrigo Felipe Rodrigues do Carmo

CPF: 073.896.164-74

Título do Projeto: DIVERSIDADE, POTENCIAL INVASIVO E IMPORTÂNCIA FORENSE DE DÍPTEROS NECRÓFAGOS EM DOIS AMBIENTES INSULARES DE PERNAMBUCO

Nome da Instituição : UFPE - UNIVERSIDADE FEDERAL DE PERNAMBUCO

CNPJ: 24.134.488/0001-08

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Táxon*	Qtde.	Tipo de amostra	Qtde.	Data

* Identificar o espécime no nível taxonômico possível.

Confirmação de Aceite do Manuscrito submetido para Acta

Zoologica 27-Apr-2017

Dear Mr. Carmo:

Manuscript ID AZ-OM-12-2016-0105 entitled "Wing morphometrics as a tool to differentiate populations of Cochliomyia macellaria and Chrysomya megacephala (Diptera: Calliphoridae) from different landscape architectures in Brazil" which you submitted to Acta Zoologica, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.

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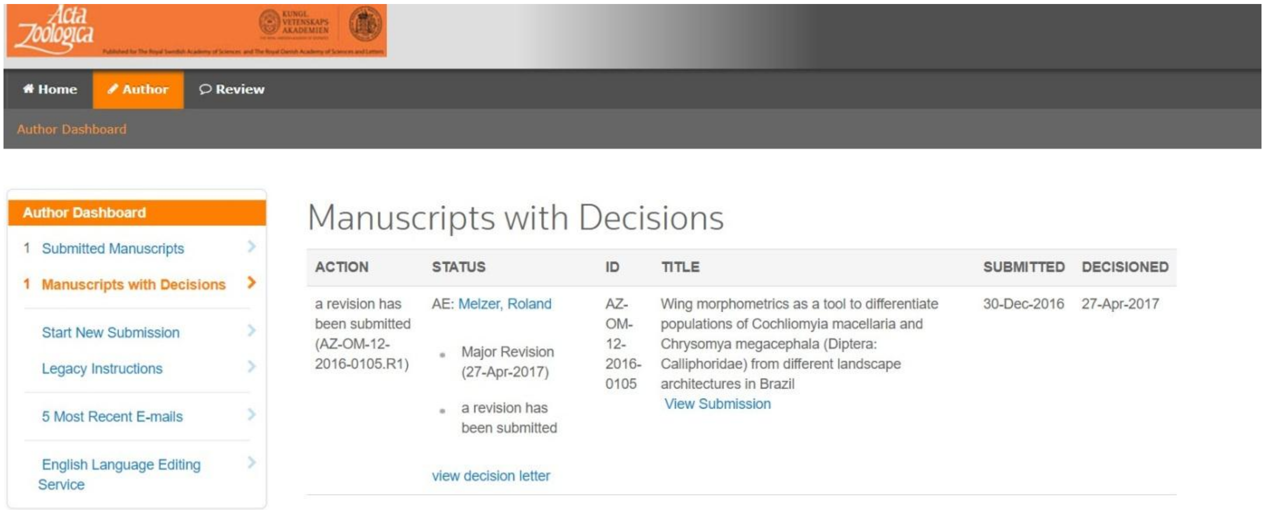

Manuscripts with Decisions

ACTION	STATUS	ID	TITLE	SUBMITTED	DECISIONED
a revision has been submitted (AZ-OM-12-2016-0105.R1)	AE: Melzer, Roland • Major Revision (27-Apr-2017) • a revision has been submitted view decision letter	AZ-OM-12-2016-0105	Wing morphometrics as a tool to differentiate populations of Cochliomyia macellaria and Chrysomya megacephala (Diptera: Calliphoridae) from different landscape architectures in Brazil View Submission	30-Dec-2016	27-Apr-2017

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Introduction.
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Papers submitted to Ecological Entomology should be original research papers on insect ecology. See Overview for more details. Full-length Original articles, Short Communications, Opinion articles and Reviews are welcomed.

Papers submitted must not have been published or accepted for publication by any other journal. Ethical considerations will be taken into account in considering the acceptability of papers, and the editors' decision on this, as on other aspects, will be final. Ecological Entomology is a member of and subscribes to the principles of the Committee on Publication Ethics.

Manuscript preparation guidelines

Examine recent issues of Ecological Entomology for details of acceptable style and format. All manuscripts should conform to the preparation guidelines. See below for guidelines specific to each manuscript type (Original articles, Short Communications, Opinion articles and Reviews).

All manuscripts should be prepared and submitted in a Word document (not PDF), double-spaced with wide margins, in A4 format. Tables must be on separate sheets, and should be self-explanatory. Figure legends should be grouped together on a separate sheet.

All papers should contain:

- An informative, concise title (up to 20 words)
- A running title (up to 40 characters)
- A self-contained abstract of fewer than 250 words presented as a series of factual, numbered statements
- Up to 6 key words
- Lines and page numbers throughout the main text
- The name, full postal address, telephone number and email address of the author to whom readers should address correspondence and offprint requests should be given on the first page (this will appear as a footnote in the journal and the publishers will send proofs to this author and address unless contrary instructions are written on the manuscript)

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All papers should be submitted online at http://mc.manuscriptcentral.com/een. Full upload instructions and support are available online from the submission site via the 'Get Help Now' button. If you are unable to submit online, please contact the Editor for details of submitting as hard copy or by e-mail.

Submission of an Original Article

Papers should be in clear concise English. Original Articles should not exceed 6000 words of text (excluding abstract and references) but longer papers of particular merit may be accepted. Papers should be novel and of wide general interest to ecologists.

Original Articles should contain the following 7 sub-headings:

- Abstract (not exceeding 250 words, including between 3 and 6 keywords)
- Introduction
- Materials and Methods
- Results
- Discussion
- Acknowledgements
- References

All Original Article manuscripts should conform to the manuscript preparation guidelines above. Manuscripts which do not conform to the standards outlined here will be returned to the author(s) with a request that they are edited to meet these standards.

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Short Communications should not exceed 1500 words (excluding abstract and references). As with Original Articles, manuscripts submitted as Short Communications should be novel and of wide general interest to ecologists. The type of material submitted as a Short Communication is flexible, for example, manuscripts might develop and/or test novel methodological techniques, might describe natural history observations, or might present new empirical or experimental data. A Short Communication should be a concise report of an independent line of research, which does not require a detailed, full-length paper.

Short Communication articles should contain the following 6 sub-headings:
- Abstract (not exceeding 250 words, including between 3 and 6 keywords)
- Introduction
- Materials and Methods
- Results and Discussion can be combined into a single section.
- Acknowledgements
- References

Furthermore, the Short Communication should include:
- A maximum word count of 1500 words
- No more than 20 cited references
- No more than 2 tables/figures

Submission of a Review Article

Invited Reviews are usually commissioned by the Editors-in-chief, or the Reviews Editor. However, non-solicited proposals for review topics are also welcome from authors and proposals should in the first instance be sent to the Reviews Editor, Klaus Reinhardt (klaus.reinhardt@tu-dresden.de). Ideas for reviews should be approximately two pages long and contain five key references. These proposals may be sent out for review before a decision is made about inviting a full Review paper. Ideas on possible review subjects have the best chance of being successful if they i) cover a current topic of general ecological interest, including evolutionary and behavioural topics, ii) have relevance to more than one insect taxon, and iii) provide a novel synthesis of current information.

Invited manuscripts should be formatted following guidelines for Original Research articles, and are peer reviewed in the same way as other manuscripts submitted to the Journal. Review articles have a word limit of 6000 words (excluding abstract and references). Reviews should contain quantitative, or semi-quantitative, analysis of published information (perhaps presented in a Table or Figure), and contain information to allow a non-expert to grasp the general significance of the topic (e.g. with a Box, Table or Figure containing background information and terminology used). Authors should consult previous Review articles for guidance.

Submission of an Opinion Article

Papers should present an original perspective on an important research-related subject of interest to the ecological community, rather than a balanced or objective review of the topic. The aim should be to stimulate debate or new research, cover controversial topics, or interpretation of, an old problem or current issue, or speculate on the implications of recent research. Please note that articles that outline recent advances in a field rather than give a strong opinion on them are not suitable for this section of the journal; neither are hypotheses without any published support.

Although subjective, an Opinion article should not be used to discuss excessively an author's own research or to criticize excessively the research of others, except where criticism is constructive.

The manuscript should be in a Word document, consist of double line spacing, wide margins and preferably in an A4 format. Tables must be on separate sheets and should be self-explanatory. Figure legends should be grouped together on a separate sheet. Manuscripts should have the same length and format as a Short Communication but sub-headings may be included at the discretion of the author and do not need to conform to a formal structure (e.g. Abstract, Methods, Results….).

Methods and Natural History Articles

Methods and Natural History manuscripts should be prepared and formatted as Original Articles (above).

Articles about methods or techniques are welcomed but only where such papers provide new and significant approaches that improve our understanding of ecological entomology.

Articles on the natural history of arthropods are welcomed but only where such papers provide new and significant information that improves our understanding of ecological entomology.

Statistics

To reduce confusion, Ecological Entomology has a standard set of guidelines for the presentation of statistical analyses. Click here for a list of commonly used abbreviations and their definitions, and these do not need to be explained in the text. However, authors must clearly state what statistical approaches were used in their analyses (supported where relevant by adequate references). Where statistics are presented in the text, we ask that the authors follow the presentation guidelines provided below.

After an analysis of variance, further simultaneous testing of treatment means should not be done, except for specific comparisons planned prior to the experiment. Simple measures of variability (e.g. SE, LSD, CI) should always accompany means. The same data should not be given in both tables and figures.

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