UNIVERSIDADE DE BRASÍLIA
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

Resposta do caracol gigante africano (*Lissachatina fulica,* Bowdich 1822) à variação climática global, regional, local e de recursos

Brasilia

2023

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Tese apresentada ao programa de Pós-Graduação em Ecologia da Universidade de Brasília, sob a orientação da Profª. Drª Rosana Tidon como critério para obtenção do título de Doutor em Ecologia

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Brasília

Janeiro de 2023

DEDICATORIA

Dedico esta tesis a mis padres, ya que marca el final de mi proceso de formación educativo. Por todas las luchas que tuvieron por más de 20 años y por todas las dificultades que lograron sortear, con la firme convicción que la educación es el bien mas preciado que uno como hijo puede heredar. ¡Gracias!

“Lo perfecto no es humano”

Mi papá

AGRADECIMENTOS

Minha gratidão com a Dra. Rosana Tidon é infinita. Ela me aceitou no seu laboratório sem me conhecer, e aceitou empreender o desafio de trabalhar com um modelo de estudo fora da sua área de conforto. Fui afortunada de ter seus cuidados, sua paciência e sua preocupação com meu processo. Estou orgulhosa da pesquisadora que virei com sua companhia. Além disso, a professora com seu exemplo me ensinou que é possível ser pessoa, mulher, mãe, filha, irmã, amiga, pesquisadora e professora neste mundo, onde quase sempre a pesquisa finaliza estragando as outras áreas da vida. Por todo o anterior, eu agradeço e espero continuar na construção da nossa sociedade.

Ao Thiago Silvester Nomiyama do Instituto Brasilia Ambiental, porque foi de extrema importância no desenvolvimento no componente de campo desta tese. Também, o Thiago virou o primeiro amigo que tive no Brasil.

Ao Dr. Alan Giraldo pela lealdade comigo neste doutorado. O Dr. Giraldo foi importante no desenvolvimento do componente teórico desta tese.

Meus colegas do Laboratorio de Biologia Evolutiva que ajudaram em campo e laboratório (Gabriel Marins, Barbara Borges). Gostaria de fazer menção especial ao Fabio Cavalcanti e a Laís Ribeiro porque ajudaram muito na minha adaptação no Brasil.

Minha família que desde Colômbia nunca duvidaram da minha capacidade e sempre estiveram alegres para mim, ainda sentiram a mesma saudade que eu. O Nestor Fabian Leyton Castro por estar sempre comigo, e todos os demais que participaram neste caminho ou que estiveram perto.

Ao PPG-Ecologia, Universidade de Brasilia e Coordenação de Aperfeiçoamento de Pessoal de Educação Superior (CAPES) pelo suporte econômico representado em materiais, instalações e bolsa necessarios para o desenvolvimento desta tese. Ao Grupo Coimbra de Universidades Brasileiras (GCUB) e a Organização dos Estados Americanos (OEA) pelo programa PAEC (edição 2018) que permitiu minha entrada ao PPG-Ecología.

Por fim, agradeço aos membros da banca por aceitarem o convite para avaliar o presente estudo.

SUMÁRIO

Resumo

As populações não nativas de uma espécie invasora devem afrontar obstáculos durante seu transporte, introdução, estabelecimento e dispersão. O clima e a disponibilidade de recursos representam alguns destes obstáculos. A resposta de populações não nativas ao clima vai depender do nicho climático realizado da espécie. Com relação à disponibilidade de recursos, a resposta vai depender de como reagem os atributos de história de vida dos organismos ante variações em recursos. O caracol gigante africano *Lissachatina fulica* (Bowdich 1822) é um molusco nativo do leste africano e classificado como invasor. Populações não nativas de *L. fulica* ocorrem ao longo da região tropical do mundo e são reconhecidas por sua ampla tolerância climática e associação com o homem. O objetivo desta tese é avaliar a resposta do caracol gigante africano (*L. fulica*) à variação climática global, regional, local e de recursos. O primeiro capitulo, mediante revisão sistemática da literatura, identifica os atributos que definem o potencial invasor de *L. fulica* e as características dos locais onde ocorre. O segundo capitulo, mediante modelagem de distribuição potencial e PCA ambiental, avalia a expansão do nicho climático realizado de *L. fulica* em função de sua dispersão pelo mundo. O terceiro capitulo, mediante analise multivariada de ordenação, avalia a densidade populacional de *L. fulica* sob diferentes condições climáticas e de intervenção antrópica na região Neotropical. O quarto capítulo, mediante trabalho de campo, caracteriza a população não nativa de *L. fulica* localizada no Distrito Federal (DF), associa sua densidade populacional, porcentagens de indivíduos mortos, com ovos, e hermafroditas, e investiga como o caracol responde a pequenas variações no clima e na presença ou ausencia de recursos. O quinto capítulo, mediante a avaliação morfométrica do crescimento de *L. fulica* no laboratório, propõe um novo método para a identificação de indivíduos hermafroditas. A revisão sistemática realizada mostrou um conhecimento fragmentado sobre os atributos de *L. fulica* e quase nulo sobre os locais onde ocorre. As informações mais estudadas foram as associadas com abundância, com pouco consenso na literatura sobre a relação da densidade populacional com o impacto. Também não há consenso na literatura sobre as classes etárias, estimadas pelo comprimento da concha, nas quais ocorre diferenciação de machos para hermafroditas. A modelagem de distribuição potencial e o PCA ambiental mostraram que o nicho climático realizado de *L. fulica* se ampliou desde sua dispersão a partir da África, em parte por sua trajetória ao longo de diferentes regiões biogeográficas. Segundo os modelos, quando o molusco saiu da Africa pode se estabelecer primeiro em áreas com climas semelhantes da área nativa na região Indo-Malaya. Quando ocorreram as introduções na região Neotropical, o molusco já se encontrava associado ao homem em climas ligeiramente diferentes. A analise multivariada de ordenação mostrou que a densidade populacional de *L. fulica* no neotropico responde ante combinações especificas de temperatura e precipitação, mas não de intervenção antrópica. Mesmo as baixas densidades populacionais, verificadas em amplas condições climáticas, são um sinal de alerta que deve ser considerado em ações de controle. O trabalho de campo com a população de *L. fulica* do DF gerou três resultados principais. Primeiro, a população não nativa no DF apresenta quase a metade dos indivíduos com potencial de pôr ovos e densidades populacionais menores ao 1 ind/m^2. Segundo, a porcentagem de indivíduos com ovos está associada à porcentagem de indivíduos hermafroditas, e em parte com a densidade populacional. Terceiro, no clima, as porcentagens de indivíduos mortos e de indivíduos com ovos respondem à temperatura máxima e umidade relativa mínima. Com relação aos recursos, os resultados obtidos em campo mostram que a densidade populacional responde à presença de solo, e a porcentagem de hermafroditas responde à porcentagem de área construída. A avaliação morfométrica da concha de *L. fulica* apontou um intervalo de comprimento onde provavelmente acontece a transição para hermafroditas e sugere dois índices morfológicos que visam identificar indivíduos que já passaram por essa transição. Em conclusão, ainda existem muitas lacunas e poucos consensos sobre o conhecimento dos atributos que caracterizam o potencial invasor de *L. fulica* e as características dos locais onde ocorre. O desconhecimento e as falta de consenso produzem falhas no manejo, já que fica difícil saber se realmente a espécie está em um ambiente semelhante da área nativa, se as densidades registradas representam algum impacto significativo ou quando pode ocorrer uma recolonização. Com esta tese se tenta preencher algumas destas lacunas. Portanto, estes resultados geram subsídios para o desenvolvimento de multiplas linhas de geração de conhecimento sobre as populações não nativas de *L. fulica*, e possivelmente para otimizar estratégias de manejo.

Palavras chave. *Achatina fulica*, história de vida, nicho climático realizado, disponibilidade de recursos, morfometria geométrica.

Abstract

Nonnative populations of an invasive species must face obstacles during their transport, introduction, establishment, and dispersal. Climate and resource availability represent some of these obstacles. The response of nonnative populations to climate will depend on the realized climatic niche of the species. Regarding resource availability, the response will depend on how the life history attributes of the organisms react to resource variations. The giant African snail *Lissachatina fulica* (Bowdich 1822) is a mollusk native to East Africa and classified as invasive. Nonnative populations of *L. fulica* occur throughout the tropical region of the world and are recognized for their wide climatic tolerance and association with humans. This thesis aimste the response of the giant African snail (*L. fulica*) to global, regional, local climate, and resource variation. In the first chapter, through a systematic literature review, we identified the attributes that define the invasive potential of *L. fulica* and the characteristics of the sites where it occurs. In the second chapter, by means of potential distribution modeling and environmental PCA, we evaluated the expansion of the climatic niche of *L. fulica* as a function of its dispersal around the world. In the third chapter, through multivariate ordination analysis, we evaluate the population density of *L. fulica* under different climatic conditions and anthropic interventions in the Neotropical region. In the fourth chapter, through fieldwork, we characterize the nonnative population of *L. fulica* located in the Federal District (DF), associate its population density and percentages of dead, egg-bearing, and hermaphrodite individuals, and describe how it responds to small variations in climate and the presence or absence of resources. In the fifth chapter, through the morphometric evaluation of *L. fulica* growth in the laboratory, we proposed a new method for the identification of hermaphroditic individuals. The systematic review carried out showed a fragmented knowledge about the attributes of *L. fulica* and almost none about the places where it occurs. The most studied information was associated with abundance, with little consensus in the literature on the relationship of population density with impact. In addition, there is no consensus in the literature on age classes, where shell length appears to differ between males and hermaphrodites. Potential distribution modeling and environmental PCA showed that the realized climatic niche of *L. fulica* has widened since its dispersal from Africa, in part by its trajectory along different biogeographic regions. According to the models, when the mollusk left Africa, it was first able to establish itself in areas with similar climates to its native range in the Indo-Malaya region. By the time of introduction into the Neotropical region, the mollusk was already associated with humans in slightly different climates. Multivariate ordination analysis showed that the population density of *L. fulica* in the Neotropics responds to specific combinations of temperature and precipitation but not to human intervention. Low population densities under wide climatic conditions are an alarm signal not to ignore these localities in the control. Field work with the *L. fulica* population in DF showed three things. First, the nonnative population in DF has almost half of the individuals with egg laying potential and population densities of less than 1 ind/m2. Second, the percentage of egg-bearing individuals correlates with the percentage of hermaphrodite individuals and in part with population density. Third, regarding climate, the percentages of dead and egg-bearing individuals respond to maximum temperature and minimum relative humidity. Regarding resources, the results obtained in the field show that population density responds to the presence of soil and that the percentage of hermaphrodites responds to the percentage of built-up area. The morphometric evaluation of the shell of *L. fulica* pointed to a length range where the transition to hermaphrodites probably takes place and suggests two morphological indices that aim to identify individuals who have already gone through this transition. In conclusion, there are still many gaps and little consensus on the knowledge of the attributes that characterize the invasive potential of *L. fulica* and the characteristics of the sites where it occurs. The lack of knowledge and consensus produces flaws in management, since it is difficult to know if the species is truly in an environment similar to the native area if the densities recorded represent any significant impact or when recolonization may occur. This thesis attempts to fill some of these gaps. Therefore, these results generate subsidies for the development of multiple lines of knowledge generation about the nonnative populations of *L. fulica* and possibly to optimize management strategies.

Key words. *Achatina fulica*, geometric morphometry, life history, realized climatic niche, resource availability.

Introdução geral

Invasão biológica

A Biologia das Invasões estuda os mecanismos ecológicos e evolutivos que determinam o estabelecimento e dispersão das espécies invasoras, com o objetivo de melhorar seu controle (Heger et al., 2021). Uma espécie é classificada como invasora quando sua presença é atribuída direta ou indiretamente ao homem, se dispersa além das barreiras biogeográficas de sua área nativa, e causa impacto (Pysek et al., 2020). O processo pelo qual passam as espécies invasoras consta de várias etapas (transporte, introdução, estabelecimento, dispersão), com diversas barreiras abióticas e bióticas (Blackburn et al., 2011). Assim, os estudos sobre invasões biológicas avaliam as respostas das espécies em localidades não nativas e, baseado nelas, quantificam impactos e aprimoram seu controle.

Espécies invasoras provavelmente se dispersam e estabelecem em localidades com climas semelhantes aos da área nativa (Wiens et al., 2010; Liu et al., 2020, 2022). A probabilidade de ocorrência de espécies invasoras em lugares diferentes da área nativa é estimada por modelagem de distribuição potencial (Liu et al., 2020). As eventuais incongruências das estimativas destes modelos têm sido recentemente atribuídas a mudanças no nicho climático realizado das espécies invasoras, produto do processo de invasão (Liu et al., 2020, 2022). Quanto aos atributos populacionais, a resposta de espécies invasoras ao clima pode ser testada mediante modelos mecanicistas ou aproximações univariadas (Gallien et al., 2010). Entretanto, há menos conhecimento sobre a resposta de atributos populacionais ao clima do que sobre padrões de ocorrência. Portanto, o clima atua como uma barreira durante todo o processo de invasão, no qual o nicho da espécie invasora determinaria seu sucesso, mas ainda há muito a se investigar sobre a sintonia fina entre o organismo e essa dimensão do ambiente.

O sucesso de uma espécie invasora na localidade não nativa pode aumentar com a disponibilidade de recursos (Sher and Hyatt, 1999) ou uma alta heterogeneidade ambiental (Melbourne et al., 2007). A heterogeneidade ambiental, definida como a variação no ambiente físico, impacta na disponibilidade de recursos (Sher and Hyatt, 1999; Melbourne et al., 2007). Mudanças nos regimes naturais de distúrbio produzidas por atividades antrópicas, como a urbanização, favorecem as espécies que podem utilizar oportunisticamente os recursos disponíveis (Sher and Hyatt, 1999; Borden and Flory, 2021). Pela alta pressão de propágulos de organismos que contam com os atributos de história de vida para aproveitar os recursos oferecidos, é esperada uma associação das espécies invasoras com as cidades (Borden and Flory, 2021). Desta maneira, o estudo dos atributos de história de vida das espécies invasoras é importante, sobretudo, para avaliar diferenças nas respostas destes frente a variações nos recursos.

A resposta das espécies invasoras ao clima e aos recursos é contexto-especifica. No clima, o javali *Sus scrofa* (Linnaeus 1758) e o mosquito *Aedes albopictus* (Skuse 1894) preencheram a maioria do clima adequavel nas regiões não nativas (Sales et al., 2017, Cunze et al., 2018). Por outro lado, alguns pássaros não nativos na Europa ocuparam só uma parte do clima adequavel nesse continente (Strubbe et al., 2013). Algumas espécies de plantas invasoras são capazes de capitalizar o aumento da disponibilidade de recursos, através do aumento do crescimento e do acúmulo de biomassa (Dawson et al., 2012). Em formigas, a disponibilidade de recursos afeta a competição. Em um ambiente competitivo (*Linepithema humile* (Mayr 1868) *x Tapinoma sessile* (Say)), *L. humile* controla o recurso de refúgio por meio de agressão, perdendo o controle sobre o recurso alimentar (Neumann and Pinter-Wollman, 2022). Portanto, o conhecimento das respostas de espécies invasoras em localidades não nativas precisa de pesquisas de história de vida em função da variação climática e de recursos.

Impactos das espécies invasoras

As espécies invasoras causam impactos ecológicos, de saúde e econômicos, dentre outros. O aumento da abundância de espécies invasoras impacta negativamente as populações de espécies nativas e suas comunidades (Bradley et al., 2019). No mundo, as espécies invasoras contribuíram no 25% das extinções de plantas e 33% das extinções de animais, enquanto as espécies nativas contribuíram em menos de 5% das extinções de plantas e animais (Blackburn et al., 2019). A saúde humana pode ser impactada por espécies invasoras mediante disseminação de infecções e introdução de novos patógenos. Por exemplo, o mosquito tigre (*Aedes albopictus*) como vector da dengue e a ambrósia comum (*Ambrosia artemisiifolia* (Linnaeus 1758)) produzindo pólen alergênico (Psyke et al., 2020). Nesse cenário, a pesquisa em invasão biológica objetiva conhecer as espécies invasoras para prevenir ou mitigar seus impactos.

Os impactos econômicos de espécies invasoras são grandes. Recentemente, a iniciativa InvaCost compilou os dados de investimento em atividades associadas com espécies invasoras (http://invacost.fr/en/accueil/). No mundo, o custo da invasão biológica representou em média 26,8 bilhões de dólares por ano (Diagne et al., 2021). Os invertebrados são o grupo onde há mais investimento (23,8 bilhões de dólares por ano), sendo 90% desse investimento para insetos (Diagne et al., 2021). Na América Central e do Sul, o custo da invasão biológica representou em média 2,2 bilhões de dólares por ano (Heringer et al., 2021). *Aedes aegypti* (Linnaeus 1758) e *A. albopictus* apresentam o maior gasto econômico na região (Heringer et al., 2021). Assim, os pesquisadores estão chamados a gerar conhecimento que melhore a eficiência do manejo das espécies invasoras.

No começo do século 21, a União Internacional para a Conservação da Natureza (UICN) criou a lista das 100 piores espécies invasoras do mundo (Lowe et al., 2000). Nessa lista há microrganismos, plantas, invertebrados e vertebrados terrestres e aquáticos (Lowe et al., 2000). Alguns exemplos de espécies são *Rhinella marina* (Linnaeus 1758) e *Myocastor coypus* (Molina 1782). *Rhinella marina* é um anuro nativo da América Central e do Sul, introduzido na Austrália em 1935 para controlar pragas agrícolas, e se tornou invasor (Phillips et al., 2007). *Myocastor coypus* é um roedor aquático nativo da América do Sul, introduzido na França para cultivo no final do século 19, e atualmente amplamente distribuído no mundo (Carter and Leonard, 2002). As espécies presentes nessa lista devem ser consideradas uma prioridade para manejo, portanto, para pesquisa.

Os gastropodes *Pomacea canaliculata* (Lamarck 1822), *Euglandina rósea* (Ferrusac 1821), e, *Lissachatina fulica,* junto com os bivalves *Mytilus galloprovincialis* (Lamarck 1819) e *Dreissena polymorpha* (Pallas 1771), estão incluídos na lista das 100 espécies invasoras mais perigosas do mundo (Lowe et al., 2000). Os gastrópodes são reconhecidos por seus impactos no ambiente e na saúde. No ambiente, *P. canaliculata* libera nutrientes na água causando eutrofização (Martins et al., 2019). *Euglandina 12ósea* foi introduzido como controle biológico de *L. fulica* e provocou a extinção de caracóis em ilhas do oceano Pacifico (Gerlach et al., 2020). Na saúde, *P. canaliculata* e *L. fulica* são reconhecidos como hospedeiros intermediários de nematódeos como *Angiostrongylus cantonensis* (Chen 1935), causador da meningite eosinofílica (Song et al., 2016). Por seus impactos, estes moluscos recebem especial atenção na pesquisa e na elaboração de planos de manejo de espécies invasoras.

Caracol gigante africano

O caracol gigante africano *Lissachatina fulica* (Bowdich 1822) é um molusco gastrópode da família Achatinidae (MolluscaBase, 2022). Essa espécie estava previamente inserida no gênero *Achatina*, mas foi transferida para o gênero *Lissachatina* por análises moleculares (Fontanilla, 2010). O molusco possui uma concha cônica com bandas de tonalidades de marrom altamente variáveis, e seu comprimento total pode atingir até 200mm (Fontanilla, 2010) (Figura 1). Como hermafrodita protândrica, *L. fulica* primeiro desenvolve o sistema reprodutor masculino e depois o feminino (Raut and Barker, 2002). Segundo seu estádio de desenvolvimento variam seus comportamentos de dispersão, reprodução e alimentação. O molusco é definido como herbívoro generalista, com mais de 500 espécies de plantas registradas como fonte de alimento (Raut and Barker, 2002). Assim, *L. fulica* se caracteriza como uma espécie com alto potencial invasor.

Figura 1. Caracol gigante africano (*Lissachatina fulica*) ativo em grama e cascalho, com o epifragma produzido no processo de estivação, e pondo ovos perto de uma parede.

Originário do leste africano, o molusco expandiu sua distribuição geográfica a partir da sua área nativa no século 19, mediado pelo homem. Durante o século 19, o caracol gigante africano se dispersou pela Ásia e Oceania por propósitos comerciais (Raut and Barker, 2002). No século 20, *L. fulica* foi registrado pela primeira vez no continente americano, e no século 21 sua presença já estava sendo registrada em diversos países desse continente (Fontanilla, 2010). Nas localidades não nativas, o molusco se caracterizou pelas abundâncias associadas com a sazonalidade. A taxa reprodutiva é mais alta com o aumento na precipitação e umidade relativa, e com uma baixa prolongada nessas variáveis *L. fulica* entra em estivação (Rahman and Raut, 2010) (Figura 1). Nos momentos de altas abundâncias podem ser registrados valores de densidade populacional até >100 indivíduos/m^2 (Gutierrez-Gregoric et al., 2011). Em quatro meses, um indivíduo de *L. fulica* pode colocar mais de 400 ovos por postura (Figura 1), com um tempo de um mês entre posturas (Roda et al., 2016; Dickens et al., 2018). Assim, o caracol gigante africano virou uma espécie invasora de importância mundial.

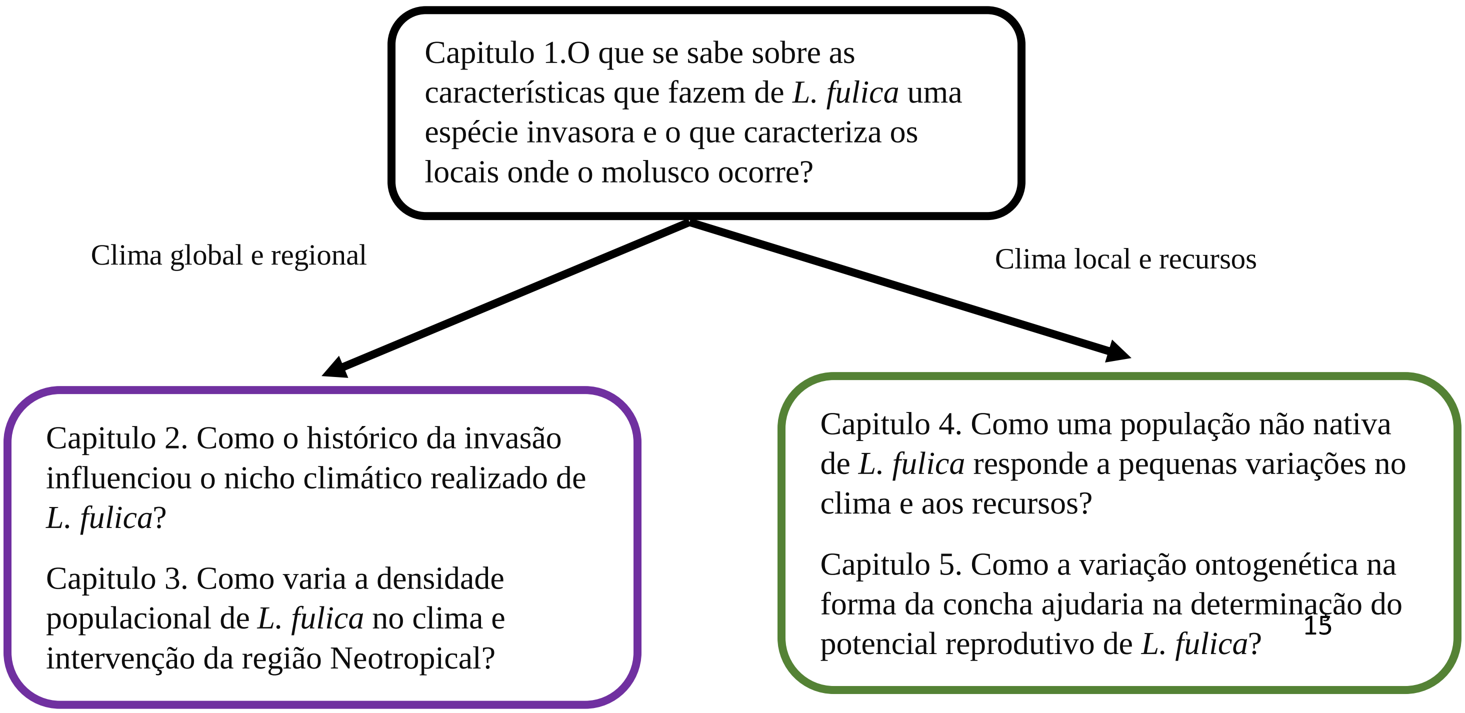
Nas áreas invadidas, *L. fulica* tem o potencial para impactar o ambiente e a saúde pública (Thiengo et al., 2007; Ramos de Souza et al., 2018). No ambiente, sua presença pode perturbar a comunidade dos moluscos locais e seus parasitas, introduzindo uma nova carga parasitaria para a comunidade nativa ou adquirindo a carga parasitaria do novo ecossistema (Prenter et al., 2004). Na saúde pública, o efeito da presença desta espécie contempla desde a incidência de nematódeos de importância medica e veterinaria (Ramos de Souza et al., 2018; Silva et al., 2022), até o incremento do pernilongo *Aedes aegypti*, principal responsável da transmissão do vírus da dengue e chikungunya (Jayashankar and Chakravarthy et al., 2014). Portanto, o controle de *L. fulica* representa um custo na ordem de milhões de reais (Smith, 2005; Jiang et al., 2022). Por essas razões, as populações do caracol gigante africano devem ser controladas, para o qual é necessário o conhecimento das respostas das populações não nativas.

Na natureza o caracol gigante africano pode ser predado por diferentes espécies, desde pássaros como o gavião caracoleiro (*Chondrohierax uncinatus*) (Martins and Donatelli, 2014), até caranguejos como *Ocypode quadrata* (Santos and Delabie, 2011). O controle intencional pode ocorrer por meios físicos, químicos e biológicos. A maneira física consiste na coleta manual com posterior sacrifício mecânico. A maneira química consiste na coleta manual ou por armadilha com posterior sacrifício com sal, cal viva, água sanitária, etc. (Raut and Barker, 2002). A maneira biológica de controle consiste no uso de predadores nativos (as vezes não nativos) do molusco (Raut and Barker, 2002; Gerlach et al., 2020). Até onde se sabe, entretanto, os predadores de *L. fulica* não são especialistas, ou seja, consomem também caracóis nativos. Portanto, conhecer a resposta de *L. fulica* ante variação climática e de recursos contribui o desenvolvimento de meios de controle sustentáveis, melhoranado assim a eficiência do manejo.

O objetivo do presente estudo é avaliar a resposta do caracol gigante africano (*Lissachatina fulica*) ante variação climática global, regional, local, e de recursos. No primeiro capítulo, mediante uma revisão sistemática da literatura, são identificados os atributos que definem o potencial invasor de *L. fulica*. No segundo capítulo, mediante modelagem de distribuição potencial, se avalia a expansão do nicho climático realizado de *L. fulica* em função de sua dispersão no mundo. No terceiro capitulo, mediante analise multivariada de ordenação, se avalia a densidade populacional de *L. fulica* sob diferentes condições climáticas e de intervenção antrópica na região Neotropical. No quarto capítulo, mediante trabalho de campo, se avalia como responde a população não nativa de *L. fulica* localizada no Distrito Federal a pequenas variações no clima e na presença ou ausencia de recursos. No quinto capítulo, mediante morfometria clássica e geométrica, se propõe um novo método de identificação de indivíduos hermafroditas (aqueles em capacidade de colocar ovos).

Perguntas de pesquisa

Como o caracol gigante africano (*L. fulica*) responde à variação climática global, regional, local e de recursos?



Capitulo 1.O que se sabe sobre as características que fazem de *L. fulica* uma espécie invasora e o que caracteriza os locais onde o molusco ocorre?

Capitulo 2. Como o histórico da invasão influenciou o nicho climático realizado de *L. fulica*?

Capitulo 3. Como varia a densidade populacional de *L. fulica* no clima e intervenção da região Neotropical?

Capitulo 4. Como uma população não nativa de *L. fulica* responde a pequenas variações no clima e aos recursos?

Capitulo 5. Como a variação ontogenética na forma da concha ajudaria na determinação do potencial reprodutivo de *L. fulica*?

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CAPITULO 1. Revisiting the worldwide invasion ecology of *Lissachatina fulica* (giant African snail): lessons learned and perspectives

Abstract

The giant African snail, *Lissachatina fulica*, one of the 100 worst invasive species in the world, is established and distributed in the tropics. The ecological knowledge about this mollusk, however, is fragmented and published in different languages. Here, we synthesize ecological research on *L. fulica* based on 100 studies from 18 countries published in English (61), Portuguese (22) and Spanish (17) between 1950 and 2020. The most studied ecological parameter was abundance, which varies broadly in space and time (< 0.01 to 150 snails/m^2). Shell length and peristome thickness have been used to estimate age structure, but there is no consensus reference regarding size intervals within each age class. Snail developmental rate and maturity are probably affected by environmental conditions and snail density. The principal variables affecting the snail distribution are pluviosity and temperature; for different scenarios, climatic models suggest slight niche expansion and increased vulnerability in the occupied areas. Finally, mortality, fecundity, population health and genetic diversity have been less explored. Snails show a wide physiological tolerance to tropical climates and consume a variety of resources. Disturbed environments such as cultivated and urban areas seem to be preferred, probably because they provide a higher availability of shelter and food resources. In conclusion, the characteristics of *L. fulica* have been more explored than the invaded locations. We propose that future research should investigate hypotheses related to propagule pressure, resource availability and biotic interactions. Standardized methodologies and improved communication among stakeholders could also support the control of this mollusk.

Keywords. Achatina fulica, Population ecology, Potential distribution, Population density, Age structure, Genetic paradox.

Introduction

Invasion ecologists have been studying the capacity of introduced species to establish, disperse and interact with other species in invaded areas (Richardson and Pysek 2008). To do so, they seek to identify the factors that make a species an invader, the ecosystem properties that determine its resistance to invasion, and from this knowledge, they develop management strategies (Huenneke et al. 1988; Richardson and Pysek 2008; Hui et al. 2016). The ability of a species to invade new areas depends on its evolutionary attributes, such as those related to survival, reproduction, and dispersal (Richardson and Pysek 2012; Hui et al. 2016). The resilience of an ecosystem to avoid invasions depends on abiotic variables, such as climate, which can act as a first filter, and on community-specific attributes, such as richness, dominance, and functional and phylogenetic diversity (Blackburn et al. 2011; Guo et al. 2015; Hui et al. 2016). Therefore, attributes of both the invasive species and the invaded environment should be studied to understand the success or failure of a biological invasion.

The giant African snail *Lissachatina fulica* (Bowdich 1822) is a gastropod mollusk ranked among the world's 100 worst invasive alien species (Lowe et al. 2004). Native to East Africa, this species is currently distributed in the tropics and subtropics (Raut and Barker 2002; Fontanilla et al. 2014). Naturalists introduced to species to India at the end of the 19th century, and the species subsequently dispersed throughout the Asian continent, Oceania, and the islands of the Pacific Ocean. On the American continent, the species was first introduced in the United States in 1966, from which it was subsequently eradicated (Raut and Barker 2002) and reintroduced in 2011 (Roda et al. 2016). At the end of the 20th century, it was recorded in Brazil and Martinique (Mead and Palacci 1992; Santana-Teles et al. 1997). Currently, *L. fulica* has been recorded in 58% of South American ecoregions (Darrigran et al. 2020), and its distribution in the Caribbean has expanded with a record noted on the island of Cuba (Vázquez and Sanchez 2014). The wide distribution of this invasive alien species has motivated scientific research in the different parts of the world where it has become established.

Most introductions and dispersal of *L. fulica* were intentional but for different purposes. In Asia and Oceania, after introduction by naturalist William Henry Benson in India, the species dispersed through commercial activities related to World War II and the postwar period (Raut and Barker 2002). In the Americas, the reasons for introduction and dispersal ranged from commercial purposes (Thiengo et al. 2007) to its use in religious rituals (Vázquez et al. 2017). In Brazil, the giant African snail was intentionally introduced for gastronomy; however, this activity was not profitable, and the snails were released and subsequently dispersed through South America (Santana-Teles et al. 1997; Thiengo et al. 2007). Finally, in Cuba, the rituals of the Yoruba religion may be a major factor in the dispersal of the snail in this country (Vázquez et al. 2017). Therefore, if human activities can influence the introduction and dispersal of *L. fulica*, they should be considered in ecological studies on the species.

Most research focused on the biology and ecology of the giant African snail presents information such as presence, abundance, population density, minimum and maximum total shell lengths, and age structure. Presence data are used in potential distribution models (Vogler et al. 2013; Sarma et al. 2015). Abundances, quantified either absolutely or as density, provide a first idea of the degree of invasion of the species (Raut and Ghara 1990). Total shell length was used for morphometric and ecological analyses (dispersal, ethology, condition factor) and to determine the age of individuals (Raut and Ghara 1990; Simião and Fischer 2004; Albuquerque et al. 2009). The frequency of total shell lengths of individuals in a population, alternatively to peristome thickness or histology, provides information on the population's age structure. The age structure allows us to infer the proportion of immatures, males, and hermaphrodites in a population (Tomiyama 1993; Simião and Fischer 2004). Therefore, although scattered, it is possible to deduce that there is enough information to answer questions about the factors that make the giant African snail a successful invasive species.

To our knowledge, the last reviews on the biology of *L. fulica* were published by Raut and Barker (2002) and Fischer (2010, in Portuguese). Since then, due to national efforts and international collaborations (e.g., Fontanilla et al. 2014; Sarma et al. 2015; Darrigran et al. 2020), there has been a large production of ecological knowledge on the giant African snail, especially in the Neotropical region, where the mollusk is widely distributed. Considering that these studies are scattered and fragmented in the literature, here, we synthesized ecological research on *L. fulica*, focusing on the species attributes and its environment. We believe that with a clearer boundary of knowledge, scientists can guide their research on established populations to avoid potential future introductions.

Methods

The literature reviews can be classified as a traditional (or narrative) review and systematic review. The systematic reviews can be classified as systematic synthesis, meta-analysis and systematic maps. The differences between the systematics reviews resides in data availability and quality. Despite being ‘gold standards’ in evidence synthesis, systematic reviews are not always feasible. High quality quantitative data is not always abundant in environmental science and methodological detail and results are often poorly reported, unreported, and/or unrecorded. Some studies are often highly heterogeneous (mixed) including different methodologies and outcomes or a mixture of quantitative and qualitative research. This may make synthesizing the results of individual studies (e.g. via meta-analysis), to answer the question, challenging or impossible. In these cases, the systematic maps answer generic questions with systematic rigor, without the quantitative demands typical of systematic reviews and meta-analyses (James et al. 2016; Berger-Tal et al. 2019).

The question that guided the present work was "What is known about the characteristics that make *L. fulica* an invasive species and what characterizes the sites where the mollusk occurs?". To answer this question, we based the document search and information analysis, whenever possible (Haddaway et al. 2020), on the systematic mapping methodology in environmental sciences (James et al. 2016) and on the ROSES (RepOrting standards for Systematic Evidence Syntheses) procedures. ROSES is a collaborative initiative with the aim of improving the standards of reporting in evidence syntheses in the field of environment. The ROSES forms for review protocols and final reports, provide detailed guidance on how to use the forms and how they were developed and published (https://www.roses-reporting.com/forms). These procedures are based on the Guidelines and Standards for Evidence Syntheses in Environmental Management, version 5.0 (CEE 2018).

We searched for peer-reviewed information in the Web of Science and Google Scholar databases, the latter in English, Spanish and Portuguese, without spatial or temporal limits. In the search, we used the keywords "*Achatina fulica*" OR "*Lissachatina fulica*" and eliminated replicated papers. We based the selection of studies on the population-outcome (PO) strategy, in which, to be included, a paper must meet the population criteria (set of individuals of *L. fulica* in any locality) and at least one of the outcome criteria (descriptor of the *L. fulica* population studied or the location where it was recorded). We undertook the selection process first by the title to meet the language (English, Spanish and Portuguese) and population (*L. fulica*) criteria. Then, we scanned the abstract to identify the main objective of the research and, finally, the full text to ensure the existence of the outcome (Online Resource 1). The documents excluded in the selection process from the abstract phase are shown in the supplementary material (Online Resource 2).

We coded a database compiling metadata and ecological information to extract information related to spatial and temporal trends in publications, attributes of *L. fulica* populations, and invaded habitats (Online Resource 3 and Online Resource 4). We used year, collection period, country, specific locality, language, journal/university, and publication type to analyze spatial and temporal trends in the literature. We described the types of studies in terms of their sampling strategies and experimental units. To evaluate the ecological data, we used a logical sequence starting from the presence, to classical population descriptors (i.e., abundance, density, age structure, eggs, total shell length, mortality, condition factor, and habitats), to information related to the molecular ecology of the species (i.e., genome type, genomic region, and genetic diversity indices studied) (Table 1). Therefore, our database comprises general metadata of the published studies, quantitative results of the populations and qualitative results in the case of habitat type. When necessary, we standardized the units of the descriptors.

Table. 1 Description of metadata and ecological information extracted from selected documents.

Variable	Description
	Metadata
Authors	Last name, First name.
Year	Year of publication.
Collection period	Period when the collection was made (month and year).
Title	Title of the study.
Journal/University	Full name of the journal or university of the study.
Type of publication	Article, abstract of scientific meeting, thesis (Ph.D., MSc).
Country	Name of the country where the study was carried out.
Specified location	Municipality/State-department-province.
Language	English, Spanish, Portuguese.
Type of study	Descriptive, experimental.
Sampling strategy	Systematic, randomized, bibliographic, opportunity.
Experimental unit	Quadrant, transect, etc.
	Ecological information
Presence	Records of individuals in localities.
Abundance	Total number of individuals collected.
Density	Number of individuals per m^2.
Eggs	Number of eggs recorded.
Total shell length	Total length of the largest shell (linear measurement from the apex to the end of the aperture).
Age description	Age classification system (length, peristome, histology).
Mortality	Percentage of dead individuals, usually calculated from empty shells.
Condition factor	Health index of the population (K).
Habitat	Type of site where individuals were found.
Genetics	Genome type (mitochondrial or nuclear), scale (complete genome or gene region), and diversity index.
Other	Parameters that were not contemplated above.

We conducted the data analysis in two stages. First, we focused on the metadata aiming to evaluate spatial and temporal trends in the literature and built a map showing the records of *L. fulica* throughout the world (GBIF 2021), and the localities were studied. In the second stage, we estimated the percentage of each ecological attribute in the database and analyzed them according to the research question. Based on this approach, we identified knowledge frontiers and possible gaps. Figures and descriptive calculations were performed in RStudio 3.6.3 software (R Core Team 2020).

We retrieved 1000 documents from Google Scholar (total base results=11200) and 833 from Web of Science. After the first filter, by title, 281 papers were selected. Of these, 181 (64.4%) were discarded after analyzing the abstract or the full text because they did not meet the criteria for language (texts in Chinese, Japanese and Russian), outcome, or because they were not available. Most papers were discarded by outcome, indicating a research bias toward topics such as parasitology, dispersal, and pest status; these issues have been widely discussed in the literature (Raut and Barker 2002; Thiengo et al. 2007; Colley and Fischer 2009; Pavanelli et al. 2017). The 100 studies retrieved to synthesize *L. fulica* ecological research were linked to Google Scholar, but only 34 were also found in Web of Science. This difference, coupled with the fact that 40% of the publications were available in Spanish or Portuguese, demonstrates the difficulty of accessing these data by the scientific community.

Spatial and temporal patterns

Studies were conducted in 18 countries, which coincided with the nonnative distribution of this mollusk (Fig. 1). India and Brazil contributed 47% of the papers (Fig. 2), which could be explained by the economic impact of this species on agricultural production (Raut and Ghose 1984; Paini et al. 2016) or public health (Silva and Morassutti 2020) in these countries. Moreover, these countries are postulated, respectively, as the first introductions outside Africa (Naggs 1997) and in South America (Santana-Teles et al. 1997), resulting in a longer research time. Therefore, the research is motivated by the presence and perceived impact of the species in their territories.

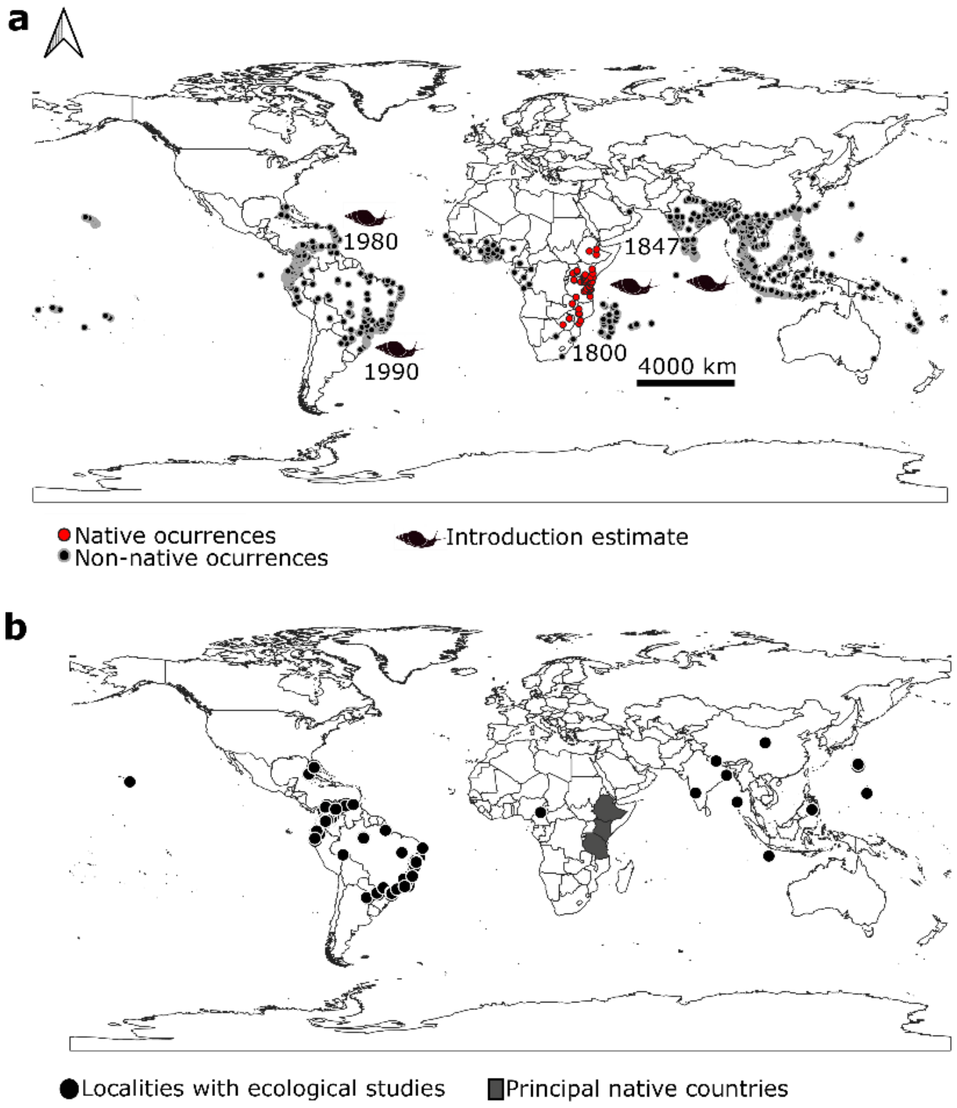

Fig. 1 Comparison between the current distribution of the giant African snail (*Lissachatina fulica*) and the localities where ecological studies on the species have been conducted. **a.** Global occurrences of the giant African snail (*Lissachatina fulica*), showing the localities in the native (red) and invaded (black) areas, together with the estimated year of introduction: 1800 outside the native area, 1847 in the Asian continent (India), 1980 and 1990 for the Caribbean and Latin American regions, respectively. **b**. Localities with records of ecological information on the giant African snail (*L. fulica*) (black dots) and native range (gray)

The studies were published between 1950 and 2020 but concentrated (87.7%) between 2001 and 2020 (Fig. 2). In general, studies published in the 20th century focused on the life history of *L. fulica*, while in the 21st century, the focus shifted to the search for control mechanisms. The increase in the number of publications after 2000, typical of biological invasion research (Richardson and Pysek 2008), is probably a product of the species being included on the list of the

100 most dangerous invasive alien species in the world (Lowe et al. 2004). However, it also coincides with the records of *L. fulica* in Latin America (Correoso and Coello 2009; Gutiérrez-Gregoric et al. 2011; De la Ossa-Lacayo et al. 2012), a region that supports six of the ten countries with the highest number of publications (Fig. 2). Regarding the collection period for the research, 50% of the studies reported less than one year, 26% reported more than one year, and 24% did not present this information. Therefore, the need for control caused by the classification of invasive alien species would be pressuring for researchers to deliver results and maintain synchrony with decision-makers (Beever et al. 2019; Requier et al. 2020).

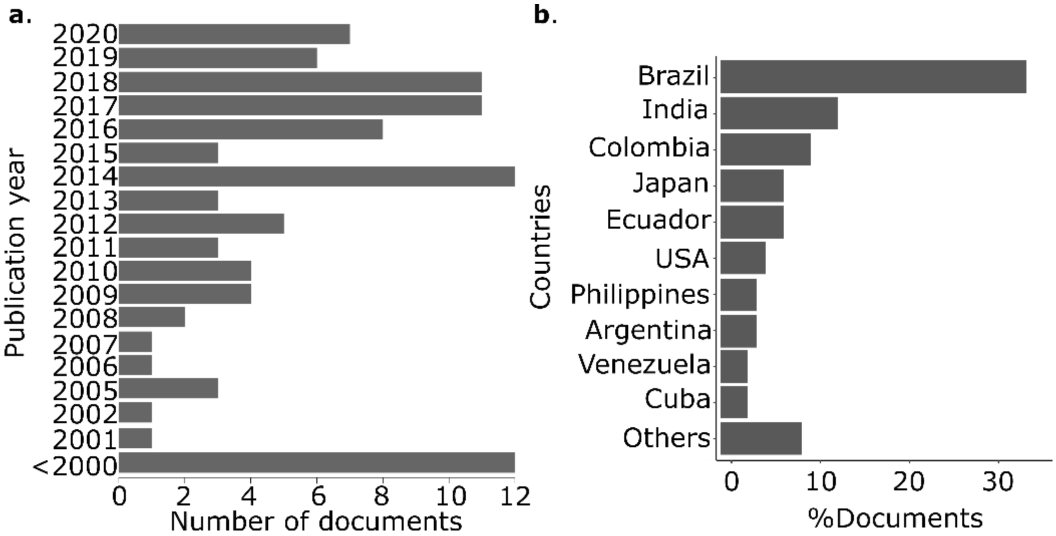

Fig. 2 Temporal and spatial distribution of ecological studies of *Lissachatina fulica*. **a**. Number of studies published per year, **b.** Percentage of papers published by country (USA=United States, others=Australia, Cameron, China, England, Mariana Islands, Nepal, Samoa, Thailand)

Characteristics of the studies

Of the 100 studies analyzed, 70% were articles, 20% were short notes, and 10% were classified into other categories (e.g., thesis, technical note, summary of scientific event). These studies were published in 74 journals, four universities, and four events. Nine journals published for 37.8% of the articles, with the most frequent as follows: PlosOne (n=5), American Malacological Bulletin (n=4), and Biota Neotropica (n=4). Regarding the type of study, 89% were descriptive, and 11% were experimental. The sampling strategy was opportunistic (43%), systematic (38%), bibliographic (7%), random (5%), 3% combined bibliographic, opportunistic, and systematic; and the remaining 4% did not clarify their sampling strategy. The most common experimental units were the individual (41%) and quadrants (27%), followed by others (26%), in which we found categories such as "Locality", "Farm", and "Group by size", and in 6% of the studies, it was not possible to determine the experimental unit.

Ecology of *L. fulica*

Among the ecological information, the most observed factors were abundance (63%), total shell length (32%), and age structure (31%), followed by population density (28%), habitat type (16%), number of eggs (12%), presence (10%), mortality (9%), genetics (9%) and condition factor (2%). Approximately 52% of the studies, in addition to the information sought, explored other types of information and life history attributes, such as shell shape, dispersal, recolonization, nest depth, predation or growth rate. This information was grouped in the category "other", as it was not included in the PO criteria. Below, we summarize the current knowledge about the ecology of *L. fulica*.

Presence

Distribution models based on the MaxEnt, GARP, and Climex algorithms generated predictions of potential areas where *L. fulica* may be established. Currently, annual cloudiness, annual precipitation, altitude, mean annual temperature, seasonality of temperature, and temperature of the coldest quarter are assumed to influence the establishment of *L. fulica*. Although *L. fulica* tolerates a wide range of precipitation (from near zero to over 4000 mm) (Adhikari et al. 2020) and temperature (from 0 to 38 °C) (Sharma and Dickens 2018), distribution models for the Neotropical region do not propose equatorial rainforests as areas of high susceptibility; instead, they propose the Cerrado region, recognized for its rainfall regime concentrated in five months of the year, as susceptible (Borrero et al. 2009; Vogler et al. 2013; Mondaini 2019). These results contrast with those found in India, whose central region shows low susceptibility to *L. fulica* establishment due to high temperatures and low rainfall (Sarma et al. 2015). Therefore, it is possible to identify differences in the predictions of *L. fulica* occurrence that would reflect the species' introduction and dispersal patterns.

The potential distribution of *L. fulica* is based on the niche conservatism hypothesis (Gallien et al. 2010; Liu et al. 2020a), i.e., models assume that the species' niche is stable in space and time and that the species has already occupied all available space (Gallien et al. 2012; Liu et al. 2020a). However, as *L. fulica* is a colonizing species, its niche could have been changed with advancement through the different stages of invasion and dispersal barriers (Liu et al. 2020a, b). Thus, *L. fulica* presents a dynamic realized climatic niche that interferes with predictions. When the Latin American models were built, the species was still introduced in some countries and established in others; in India, on the other hand, the mollusk populations of this species were already established when the model was developed. The ability of models to predict areas of occurrence should be improved with new datasets (Yates et al. 2018; Liu et al. 2020b), preferably on a global scale and with occurrences in the exotic distribution of interest (Vogler et al. 2013; Sarma et al. 2015) or the native and exotic distributions of interest (Mondaini 2019).

For the future, potential distribution models based on the CMIP5 (Coupled Model Intercomparison Project-Phase 5) climate change scenarios report little expansion of the species' climate niche and increased vulnerability of occupied areas under both the optimistic (RCP2.6) and pessimistic (RCP8.5) scenarios. However, it should be noted that these models were applied only to data in India (Sarma et al. 2015) and Brazil (Mondaini 2019), without considering records of the species on a global scale. Climate change brings consequences to invasive species, such as changes in the distribution, impacts, and effectiveness of control strategies (Hellmann et al. 2008). Therefore, there is still a long way to understand the effect of these changes on the distribution of *L. fulica*, and future work should explore this topic.

Climate and physiology

Potential distribution models are generally based on rainfall and temperature variables, which regulate physiological processes such as estivation and reproduction. Under conditions of low rainfall and high temperatures, snails experience estivation. This mechanism allows establishment at the population level, but for the individual, it leads to losses of weight, sugar, and ions such as magnesium and potassium (Rahman and Raut 2010; Rahman and Raut 2012). Estivation is mainly triggered by low relative humidity (<40%), and its timing is regulated by food availability and individual size (measured as total shell length) (Rahman and Raut 2010; Rahman and Raut 2012). In terms of reproduction, the optimal conditions for mating, egg laying, and hatching include high relative humidity and temperature between 22 °C and 32 °C (Raut and Ghose 1984; Sharma and Dickens 2018). Thus, even in regions with long periods of drought, such as the Brazilian Cerrado and India, snail populations can become established by synchronizing their feeding and breeding activities with the rainy seasons.

Abundance and density

To determine the magnitude of the *L. fulica* problem, most of the studies recorded the number of individuals observed or collected. In the 100 papers analyzed, 127,339 mollusks were counted. The population density was recorded in 35 localities, and the majority corresponded to less than 10 ind/m^2. The range of density values was high, oscillating between 0.00015 ind/m^2 in Havana, Cuba (Vázquez and Sánchez 2014), and 150 ind/m^2 in Andrés Bello, Venezuela (Herrera et al. 2016). It should be clarified that variation in abundance and density values could reflect different sampling times and strategies. In general, these population attributes are strongly associated with humidity (Gutiérrez-Gregoric et al. 2013) and food resources (Fischer et al. 2008). Different values have been proposed as thresholds to recognize the potential impact of the exotic population, such as 10 ind/m^2 (De La Ossa et al. 2017), 30 ind/m^2, or even 100 ind/m^2 (Almeida 2018). According to these thresholds, *L. fulica* would not represent a real problem in most localities. However, these conclusions should be considered with caution because the relationship between density and impact is not linear (Jackson et al. 2015).

Density-impact curves allow estimating the density range in which a species causes more impact and the type of control to be carried out (e.g., prevention, mitigation, containment, eradication) (Green and Grosholz 2021). Therefore, it is an effective tool for environmental authorities to control species while optimizing the use of resources (Green and Grosholz 2021). However, the values found in the different studies lack support, as the density-impact relationship can be affected by the trophic level of the invasive species and can differ according to the assessed impact (Jackson et al. 2015; Bradley et al. 2019). Therefore, to estimate the density-impact curves of *L. fulica*, it is necessary to investigate the temporal and spatial factors involved in the variation in density and impact indicators of *L. fulica* based on its life history.

Shell morphology

The shell morphology of *L. fulica* can be described by linear measures of the whole shell, of its aperture, or by geometric morphometrics. Shell length is considered the most biologically relevant measure because its growth rate is higher than that of the other measures (Avendaño and Linares 2015; Santos et al. 2020). This measure is proposed as an age estimator because it shows constant growth in at least the first 12 weeks of life and then decreases (Vázquez et al. 2018). It is estimated that the total shell length can reach 300 mm (Vinci 1998; apud Albuquerque et al. 2009), but there is no record of these individuals in the field. In the field, the largest shell lengths ranged from 39 mm (Sucre, Colombia) (De la Ossa et al. 2017) to 200 mm (Minas Gerais, Brazil) (Barçante et al. 2005). In addition to age, the measurement of length has become the standard independent variable when the objective is to establish relationships with the other information of *L. fulica*, such as density (Raut and Ghara 1990) and weight (Albuquerque et al. 2009).

Shell shape has been evaluated by geometric morphometrics, revealing greater intrapopulation than interpopulation variation and a possible variation in shape with centroid size (size descriptor) (De la Rosa et al. 2010; Sobrepeña and Demayo 2014a,b). Accordingly, these patterns could indicate a change in shape throughout ontogeny. Regarding color patterns, research is more limited, as the mechanism responsible for shell pigmentation in gastropods is poorly known. In summary, knowledge of *L. fulica* shell morphology is relatively extensive in terms of classical morphometrics when compared to geometric morphometrics and color patterns. Whether shell characteristics are related to ontogenetic development or environmental conditions is still an open question that future studies should explore.

Eggs

The number of reported eggs per clutch varies between 85 (Fischer and Colley 2004) and 460 (Roda et al. 2016). Egg number, volume, and viability are positively associated with shell length (Tomiyama and Miyashita 1992; Roda et al. 2016). Egg production increases with shell size until a limit at which it begins to decrease (Roda et al. 2016). Additionally, an increase in population density negatively affects egg number, viability, and age at first reproduction (124 to 170 days) (Dickens et al. 2018a, b). Because of these associations, we recommend that in addition to counting the number of eggs in the field, egg volume and viability should be estimated. Finally, it is important to inform whether eggs were counted per individual or population, especially when there is the potential for self-fertilization (Dickens et al. 2018b).

Age structure

The age structure of *L. fulica* has been used as an indicator of population status. The presence of all age groups is interpreted as a sign of an established population, while the predominance of young individuals suggests a recent or declining population (Avendaño and Linares 2015). This information is essential for researchers and decision-makers, as snail behavior, dispersal, and resource consumption vary among age groups. For example, smaller individuals migrate more (Raut and Ghara 1990; Fischer et al. 2012), and larger individuals exhibit homing behavior (Tomiyama 1992, 1994, 2002), which influences their home range (Cuasapaz and Salas 2019). Therefore, determining the age of individuals in a population facilitates our understanding of ecological processes and informs decision-makers on reproductive and dispersal potentials.

Estimates of age structure are usually based on size classes or peristome thickness. The first attempt to group *L. fulica* individuals by age was found in Nishida and Napompeth (1975), who constructed a life table classifying individuals by weeks based on the logistic growth equation. Subsequently, Tomiyama (1993) used peristome thickness as a criterion for estimating the age of individuals. It was shown that growth is greater and peristome thickness is lower in young adult males than in hermaphrodites. Based on these data, Tomiyama (1993) proposed classifying individuals with peristome thickness greater than 0.5 mm as males and greater than 0.8 mm as hermaphrodites. However, it should be noted that the relationship between the peristome and egg laying is inconclusive (Dickens et al. 2018b). Currently, the transition from juvenile to functional male is considered to occur in specimens of approximately 40 mm. The size of the snail during its transition to hermaphrodite is not yet agreed upon; there are three proposals regarding the total shell length at this time: 60 mm (Colley and Fischer 2009), 70 mm (Simião and Fischer 2004), and 90 mm (Sá-Oliveira et al. 2016).

Some studies have attempted to determine the total shell length interval at which individuals develop the female reproductive system. Tomiyama (1993), based on histological analysis, estimated the mean length of males at 53.8 mm ± 7.21 mm and that of hermaphrodites at 61.8 mm ± 6.26 mm. Vázquez et al. (2018), through laboratory monitoring, established hermaphroditism at 22 weeks or 70 mm in length in individuals from Cuba; however, gravid individuals have been found in the field as early as 48 mm in the United States (Roda et al. 2016). Therefore, there is a gap in determining the age and reproductive developmental stage of individuals in *L. fulica* populations, making it difficult to study the ecological processes of the species.

The differences found in reproductive development can be explained by resource availability, rainfall seasonality, or control. Resource availability and rainfall seasonality affect the estivation period, which, depending on the conditions, can reach 11 months (Raut and Ghose 1984). During this period, there is a decrease in metabolism that affects the growth of the individual (Nishida and

Napompeth 1975; Rahman and Raut 2010). Control, however, systematically eliminates larger individuals, as they are more detectable. Decreasing the frequency of hermaphrodites in a population, in turn, can accelerate the maturation process of the reproductive organs of young individuals, as has been reported in other gastropod species under similar pressures such as hunting activities (Tewfik et al. 2019). A better understanding of the factors influencing the reproductive development of *L. fulica* populations should improve models aimed at inferring its age structure and, consequently, its management.

Mortality and condition factor

In the laboratory, the species showed a type 1 survival curve, i.e., mortality was higher in senescent individuals (Vázquez et al. 2018). In the field, mortality rates ranged from 1% (Sá-Oliveira et al. 2012) to 97% (Lake and O'Dowd 1991). However, variations in mortality rates were explored only in the study by Lake and O'Dowd (1991), who recorded higher mortality percentages in an undisturbed habitat than in a disturbed habitat, possibly due to predation by a native crab (*Gecarcoidea natalis*). Thus, little is known about which biotic (e.g., predation occurs in the cases of *Chondrohierax uncinatus* (Ramírez-Jaramillo 2018), *Leptodactylus ocellatus* (Santana and Batalla 2018), and *Ocypode quadrata* (Santos and Dellabie 2011)) or abiotic factors regulate mortality. Therefore, there is less information on factors influencing the mortality of *L. fulica* than influencing its natality (see eggs and age structure).

In addition to mortality, an understudied parameter is the condition factor (K). This parameter estimates population health based on the allometric relationship between the length and weight of individuals, corrected by the allometric coefficient (b) of the population-specific equation. Central tendency values for this factor were calculated at two locations (mean = 0.0006 and median = 0.97) (Albuquerque et al. 2009; Almeida 2014). However, it is unclear how Fulton's K can be interpreted in mollusks, as the interpretation of Albuquerque et al. (2009) was derived from fish. Despite the difficulty of interpretation, assessing the health of *L. fulica* populations may be of interest to understand their relationship with the environment and establish control strategies.

Habitats

The giant African snail is found in habitats with different degrees of anthropogenic intervention. In cities, it can be found in places that offer shelter, such as walls, vacant lots, gardens, courtyards, and religious centers (Albuquerque et al. 2008; Gutiérrez-Gregoric et al. 2013; Vázquez et al. 2017). The species has also been recorded in preserved and disturbed forests (Lake and O'Dowd 1991; Oda et al. 2020), being more abundant in the latter, probably due to a lack of biotic resistance (Lake and O'Dowd 1991). This versatility suggests a high adaptive capacity of the mollusk to establish itself in new locations. However, further studies are needed to assess the association between the population parameters of the species with the intervention gradients or types of disturbance.

Habitats in which *L. fulica* occurs are characterized by a wide range of food resources. Agricultural or ornamental crops provide plant species such as *Ananas* spp., *Annona muricata*, *Brassica oleracea*, *Caffea arabica*, *Capsicum annuum*, *Carica papaya*, *Citrus* spp., *Hylocereus undatus*, *Lactuca sativa*, *Malpigia emarginata*, *Mangifera indica*, *Manihot esculenta*, *Musa paradisiaca*, *Musa acuminata*, *Passiflora edulis, Panicum miliaceum, Persea americana, Phaseolus vulgaris*, *Psidium guajava*, *Punica granatum*, *Theobroma cacao, Saccharum officinarum*, *Solanum tuberosum*, *Solanum lycopersycum*, *Vitis vinifera*, *Vitis labrusca,* and *Zea mays*, among others (Raut and Ghose 1984; Fischer and Colley 2004; Goldyn et al. 2016; Aguilera and Ortiz 2020). In cities, this mollusk species has been reported to consume inorganic matter and animal feces, and in Hawaii, it has been observed to prey on slugs (Meyer III et al. 2008). Therefore, the giant African snail would be better classified as a generalist than as an herbivore, and this generalism certainly facilitates its establishment.

Borden and Flory (2021) proposed that selection pressures characteristic of urban areas may enhance the attributes of invasive species. In this context, populations of *L. fulica* occupying urban or cultivated areas may adapt to these disturbance conditions (Fischer et al. 2012). Similarly, disturbances in forests (such as the introduction of alien species) (Green et al. 2011) and cultivated agricultural areas provide opportunities for the establishment of *L. fulica*. As a result, potential economic and environmental impacts such as those that occurred in India may appear. In this context, we recommend studies investigating the effect of different gradients of anthropogenic disturbance on *L. fulica* population parameters; such an approach seems promising for identifying potential selection pressures acting on the giant African snail.

Genetics

For *L. fulica*, there are studies at the metagenomic, genomic, chromosomal, and gene levels. In the metagenome associated with the snail, there is a high diversity of bacteria carrying genes responsible for decomposing cellulose and hemicellulose, indicating potential in the biofuel industry (Cardoso et al. 2012). At the genomic level, the nuclear (Guo et al. 2019) and mitochondrial (He et al. 2014) genomes have already been characterized. With 2.12 Gb and high repetitive content in the nuclear genome, populations with different haploid chromosome numbers (30 in India and 31 in China) are recognized (Thiriot-Quiévreux 2003; Guo et al. 2019). For the mitochondrial genome, 15507 bp and 37 genes were counted, with a possible new position of the control region within Stylommatophora (He et al. 2014). In addition to chromosome number, the populations differ in terms of karyotype, with individuals evaluated in India having more metacentric chromosomes (28 m, 2 sm) and those in China having more telocentric chromosomes (13 m, 1 sm, 17t) (Thiriot-Quiévreux 2003). At the gene level, sequences and genetic markers have been used to assess the global dispersal pattern of this mollusk species (Fontanilla et al. 2014). For these reasons, *L. fulica* represents a promising model organism to investigate molecular, evolutionary, and ecological phenomena in gastropods, broadening the base of genomic research in the group (Gomes dos Santos et al. 2020).

In omics approaches (e.g., whole genome, mitogenome, metagenome, and transcriptome), sequences are known, but they have not been applied to answer ecological questions. For example, the metagenome of *L. fulica* may provide information to assess the role of the species' microbiome in its feeding, resource utilization, reproduction, and development (Chalifour and Li 2021). Transcriptome information, using RNA-seq and SNPs, can help explain the varying success of exotic populations in different habitats, despite having a similar gene pool (Lamar et al. 2020). In summary, genomic tools can contribute to a deeper understanding of the processes involved in the adaptive genetic variation of *L. fulica*, from its introduction into new areas to its establishment and subsequent dispersal.

Genetic sequences and molecular markers have been used to study the patterns of introduction and dispersal of *L. fulica*. In general, low genetic diversity has been found in exotic populations. Initially, 18 haplotypes of the mitochondrial 16S gene were found, with a haplotypic diversity of 0.797 and a nucleotide diversity of 0.012 in native localities and values of 0.205 and

0.001 in invaded localities, respectively. The most widely distributed haplotype is C, and haplotypes that are different from those of the native area are found in nonnative localities, such as Q in Ecuador and S in India (Fontanilla et al. 2014; Ayyagari and Sreerama 2017). Other gene regions, such as the COI gene (Woogeng et al. 2017; Dumidae et al. 2019, Vijayan et al. 2020) and microsatellite molecular markers (Morrison et al. 2014; Patiño-Montoya and Giraldo 2017), provide evidence of low genetic diversity at the local scale. Therefore, the results of these studies point to an apparent paradox, as exotic populations of *L. fulica* are well-established despite low genetic diversity (Estoup et al. 2016).

A paradoxical population is defined by having experienced a strong founder effect with subsequent establishment and dispersal (Estoup et al. 2016). Until this review, all molecular studies of *L. fulica* have used few neutral markers (primarily mitochondrial regions) suitable for observing genetic variation that is theoretically not under selection (Dlugosch et al. 2015). The use of these markers does not consider much of the genetic variation in the species, the effect that loci have on the phenotype, the occurrence of cryptic variation after invasion, and the new genotypic combinations that may arise in cases where there are multiple introductions (Dlugosch et al. 2015). Considering that current knowledge on genetic variation in *L. fulica* focuses on neutral variation, i.e., does not consider the other expressions of genetic variation, it is still too early to be sure that a paradox exists in *L. fulica* populations (Dlugosch et al. 2015; Estoup et al. 2016).

Limitations

The main limitations for the development of this study were the low availability of older literature and the lack of methodological standardization. We could not access part of the literature cited before 2000, and the lack of methodological standardization prevented us from performing more in-depth analyses. For example, the quadrant-based collection method for ecological purposes was proposed by Craze and Mauremootoo (2002), followed by some authors (Albuquerque et al. 2009; Sabino et al. 2014); in most of the literature, however, there is no methodological standardization. This lack of standardization and time investment in sampling hinders the elucidation of macroecological patterns or the recognition of how the attributes that make *L. fulica* an invasive species interact with abiotic and biotic factors.

Conclusion and perspectives

The characteristics that make the giant African snail an invasive species are its broad climatic tolerance, generalist feeding habits, and high reproductive capacity. The wide tolerance of temperature and precipitation allowed its establishment in the tropics, and according to potential distribution models, there may be an expansion of the species' realized climatic niche. The generalism of the species is demonstrated by records of slug predation, consumption of vegetables, animal feces, and inorganic matter. In reproduction, maturation of the female reproductive system occurs in individuals with different shell sizes, which probably corresponds to individuals of different ages. Differences in maturation periods among populations would be associated with resource quality or control actions. Therefore, the high invasive potential of the giant African snail qualifies it as one of the 100 world´s worst invasive alien species.

The sites where the giant African snail occurs are mainly characterized by an availability of food and shelter. However, most studies have focused on abiotic characteristics, such as temperature and rainfall. Knowledge of how populations are associated with gradients of anthropogenic intervention or how they interact with native species is still very superficial. Thus, new lines of research should explore the snail-environment relationship at a fine grain level (i.e., urban heterogeneity, genic microstructure, interspecific relationships at the microhabitat level) to investigate its ecology and to develop control strategies. To this end, current methodologies should be standardized and updated according to the needs of research gaps through collaboration between researchers from different countries.

The wide distribution of the giant African snail, combined with its life history characteristics, makes it a good model for testing hypotheses related to biological invasions. For example, Lake and O´Dowd (1991) addressed the hypothesis of biotic resistance and provided the starting point for studies associating *L. fulica* with urban centers (Albuquerque et al. 2008, 2009). Likewise, research based on hypotheses related to biotic interactions, resource availability, or propagule pressure (Enders et al. 2020) could support new questions and answers that clarify ecological processes. To investigate the hypotheses of biotic interactions and resource availability, however, it is necessary to improve the distribution models that increase our understanding of the realized and fundamental niches of *L. fulica*. Furthermore, to explore the effect of propagule pressure, it is necessary to advance genetic sampling and incorporate genomic tools. Therefore, future ecological research with *L. fulica* must be interdisciplinary and have the clear objectives of identifying the factors that make the snail invasive and the properties of the places where it is found, thus helping to develop management strategies.

The application of ecological knowledge of *L. fulica* in management strategies is still problematic and should be improved in the future. The problem starts with insufficient communication among stakeholders (such as scientists, citizens, decision-makers), which affects the production and dissemination of ecological research. Especially in Neotropical countries, improving communication in society can avoid biases due to pressure for quick results and increase the scientific community's efforts to solve problems. Adequate communication among interested partners will make it much easier for researchers to solve the methodological standardization problem found in this work. With a consensus on methodologies, the works would be easier to perform and compare with each other, leading to an improvement in the interpretation of ecological patterns at different spatial and temporal scales. In addition to the above, the effort to fill the information gap about native populations would benefit from good communication and methodological standardization, providing a reference point for decision-making regarding control measures. By advancing on these points, researchers would be motivated to go beyond studies with immediate and local results, posing deeper questions and hypotheses that may arise through international collaboration. Thus, the production of ecological knowledge of *L. fulica* can contribute both to the theory of invasion ecology and to the implementation of comprehensive management strategies.

Acknowledgments

We thank the Coordenação de Aperfeiçoamento de Pessoal de Educação Superior (CAPES) for the first author's doctoral study grant (CODE 001) and MCTIC/CNPq/FAPEG (grant 465610/2014-5) for financial support.

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CAPITULO 2. Effect of the invasion history of the giant African snail (*Lissachatina fulica*) on its realized climatic niche

Paper published in Invertebrate Biology accepted in September 2 2022

Available online October 28 2022

Patiño-Montoya, A., Giraldo, A., & Tidon, R. (2022). Effect of the invasion history of the giant African snail (*Lissachatina fulica*) on its realized climatic niche. *Invertebrate Biology*, e12385.

Abstract.

There is almost a century of difference among Indo-Malayan, Australasian, and Neotropical regions in establishment of non-native populations of the giant African snail (*Lissachatina fulica*). Using potential distribution models and environmental principal components analysis (PCA-env), we first tested whether an expansion of the realized climatic niche of *L. fulica* occurred. The models showed geographical differences between the native and non-native areas, especially in the Neotropical region, where the last introduction of molluscs occurred. Because PCA-env showed a 60% expansion and 40% overlap between the native and global areas, we next investigated whether the expansion of the realized climatic niche of *L. fulica* was influenced by its geographical spread. Precipitation had the highest contribution in most models, but temperature was the variable that best explained the projected spread from the current Neotropical distribution. The current Neotropical distribution was better explained when the climatic conditions of the Indo-Malayan and Australasian regions from which the species arrived in the Neotropics were included. PCA-env showed 74% expansion between the native and Indo-Malayan–Australasian areas and 97% expansion between the native and Neotropical areas. In conclusion, the spread between biogeographic regions and the existence of similar climatic conditions between the native and non-native distributions would produce the observed climatic niche of *L. fulica*.

Keywords**.** *Achatina fulica*, invasive mollusc, niche expansion, niche overlap, niche stability.

Introduction

Invasive alien species occur beyond the limits of their normal geographic ranges, are often transported by human actions, and impact the economy, environment, and health (Pyšek et al., 2020). When dispersed outside their native areas, invasive species experience a series of stages (e.g., transport, introduction, establishment, and dispersal) that comprise biotic and abiotic barriers, such as enemies and stressful climates (Blackburn et al., 2011). While overcoming these barriers, successful invaders exhibit phenotypic plasticity (Davidson et al., 2011) and genetic modifications that counteract the negative effects of ecological and evolutionary processes (Dlugosch et al., 2015). Therefore, invasive species in non-native areas can change their fundamental and realized niches, and this concept should be considered when estimating the probability of invasion in a given region (Guisan et al., 2014; Pearman et al., 2008; Sales et al., 2017; Václavík & Meentemeyer, 2012).

Species distributions can be depicted as gridded projections in which each spatial unit (grid cell) has a value of *n* environmental features at a given time (Colwell & Rangel, 2009; Sales et al., 2021). The niche, defined as the intersection between favorable biotic and abiotic environmental conditions and the dispersal abilities of a species (Soberon & Peterson, 2005; Sales et al., 2021), can be classified as either fundamental or realized. The fundamental niche, which encompasses all conditions under which species can survive and reproduce, differs from the realized niche, which represents the conditions in the observed distribution of species in the physical world (Colwell & Rangel, 2009). Over time, the realized niche can be modified by dispersion, adaptation, local extinctions, and species interactions, without any change in the fundamental niche (Colwell & Rangel, 2009). This theory is useful for applications in invasion biology, such as establishing potential areas for invasion or identifying differences in conditions between native and non-native areas.

Niche conservatism refers to the tendency of a species to retain its ecological attributes over time (Wiens et al., 2010). Biological invasion implies that organisms face new climatic conditions, and niche conservatism in invasive species is a challenge (Guisan et al., 2014; Liu et al., 2020a). Although it is expected that the success of a species in establishing itself is greater when it is introduced in places climatically similar to its native area (Liu et al., 2020a), different niche changes during geographical expansions have been reported. Among birds, the evaluated species occupy only a part of the suitable environments in the non-native part of the range (Pili et al., 2020). In amphibians, different species show climatic niche shifts, niche conservatism, or environmental nonequilibrium in native or non-native parts of the range (Strubbe et al., 2013). These differences have led to a debate in the literature regarding the difficulty faced by potential distribution models in predicting the susceptibility of areas to invasion (Pili et al., 2020; Václavík & Meentemeyer, 2012). Thus, the absence of niche conservatism in an invasive species would be a consequence of a change that occurs owing to the release from limitations in its native area, introduction type, or establishment time (Li et al., 2014; Liu et al., 2020a).

The giant African snail *Lissachatina fulica* (BOWDICH 1822) is a gastropod mollusc classified as one of the 100 worst invasive alien species in the world (Lowe et al., 2000). The species was first classified in the genus *Achatina* and later transferred to *Lissachatina* using a molecular approach (Fontanilla, 2010). This mollusc is native to eastern Africa and was introduced to Madagascar and the islands of the Indian Ocean in the first half of the 19th century. From Madagascar, the species spread throughout Asia and Oceania (Raut & Barker, 2002) in the Indo-Malayan and Australasian biogeographic regions (Olson et al., 2001). The first introduction of the snail to America occurred in Florida (USA) in the first half of the 20th century. The snail was then eradicated, but a new introduction occurred in the 21st century (Roda et al., 2016). In the second half of the 20th century, introductions from Indonesia were recorded in the Caribbean islands and Brazil; since then, snails have been spreading in Neotropical regions (Darrigran et al., 2020; Olson et al., 2001; Thiengo et al., 2007). Despite the environmental differences among the three biogeographic regions where the snail occurs, it is unknown whether the invasion chronology of *L. fulica* has had any impact on its climatic niche.

Assuming that the current worldwide distribution of *L. fulica* is the result of a long invasion process, we tested the hypothesis that the species' realized climatic niche has expanded. Because this first hypothesis was supported by the worldwide correlative potential distribution model and native and global environmental principal component analysis (PCA-env), we next investigated whether the expansion of the realized climatic niche of *L. fulica* was influenced by the spread from its native to the non-native areas (Africa to Indo-Malaya and Australasia to Neotropics). Expansion of the realized climatic niche in *L. fulica* could have occurred as it spread through the Indo-Malayan and Australasian regions because these regions include climatic conditions both similar and different from those of its native area. This change in climatic conditions, in turn, could explain the ability of species to continue spreading into areas with new climatic conditions, such as in the Neotropics. We evaluated this second hypothesis using a correlative potential distribution model to obtain possible differences in geographic space and a PCA-env approach to estimate the expansion at each invasion step.

Methods

Records of *Lissachatina fulica*

Localities where the giant African snail (*L. fulica*) is present were initially obtained from the Global Biodiversity Information Facility (GBIF) database (GBIF, 2021) and complemented with localities reported in Vijayan et al. (2020), for an initial total of 5086 records. The records were subjected to three filters: the elimination of duplicate records, elimination of points without established populations (oceans and Europe; in the case of Europe, there are records of introductions of the species, but there is no evidence of successful establishment), and reduction in spatial autocorrelation. To avoid spatial autocorrelation, the Moran index was used to exclude points less than 5 km apart, with a cut made using the spThin package (Aiello-Lammens et al., 2015) of the RStudio 4.0.1 program. Ultimately, 1657 valid records were retained throughout the current distribution of this species. These records were grouped by biogeographic region according to the classification of Olson et al. (2001). The localities between longitudes 39.666 and 153.062 were grouped in the Indo-Malayan and Australasian regions (n = 949), and those between longitudes -108.388 and -33.013 were grouped in the Neotropical region (n = 469). The native part of the range corresponds to localities in eastern Africa (n = 66; total number of African localities, n = 131) (Raut & Barker, 2002). The localities corresponding to the islands of the Pacific Ocean (n = 108) outside the biogeographic regions were classified separately and only used in the current to global projection model, as were the African localities that did not correspond to the native area (Figure 1).

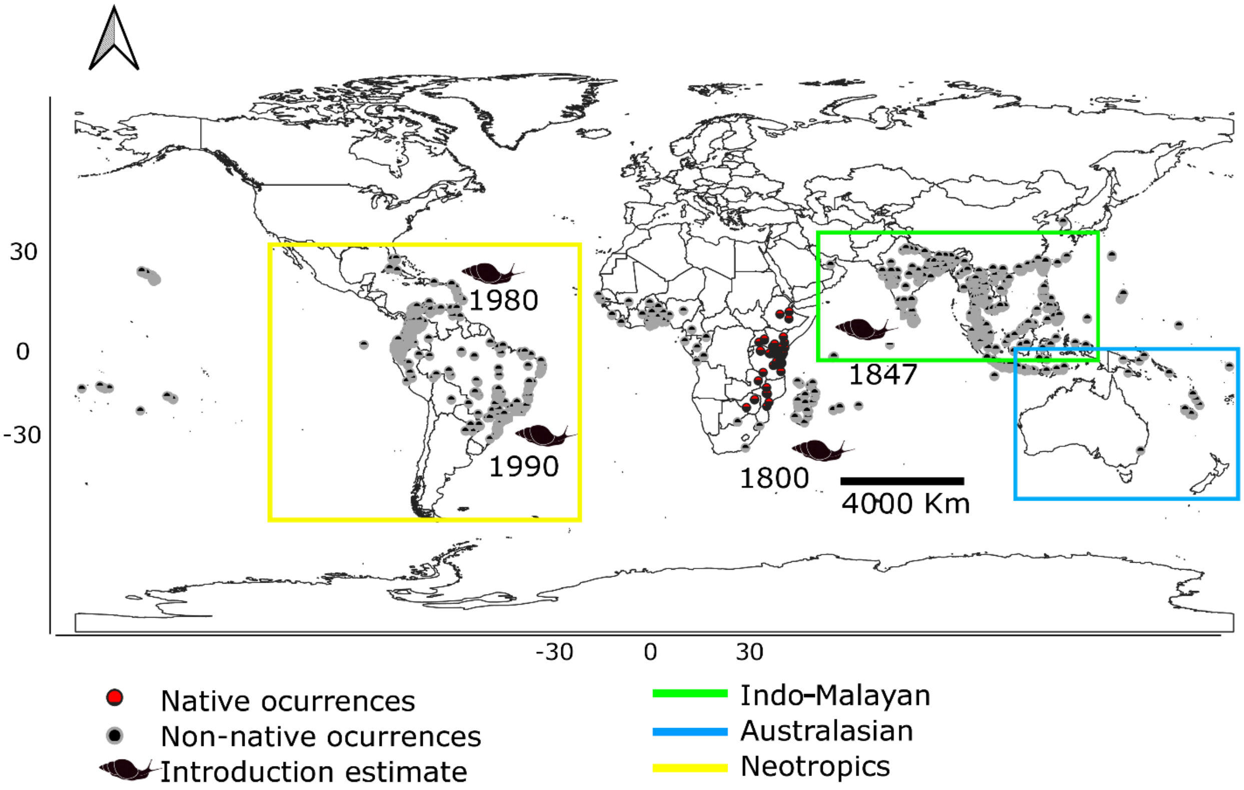

Figure 1. Localities of the giant African snail (*Lissachatina fulica*) within its native (red dots) and non-native areas (black dots). For non-native areas, the Indo-Malayan (green rectangle), Australasian (blue rectangle), and Neotropical (yellow rectangle) biogeographic regions are indicated, as well as the approximate year of first introductions.

Climate data

The climate variables used in the models were the current climate layers in the WorldClim platform, with a resolution of 5 km (i.e., 2.5 arcminutes) (https://www.worldclim.org/data/worldclim21.html). These variables were derived from the monthly values of global temperature and precipitation during a time series of 30 years (1970–2000) and were created to identify biologically important variables (Table 1). For the selection of climatic variables, multicollinearity was evaluated using Pearson correlation (Dormann, Elith et al., 2012). When a pair of variables had a Pearson's correlation coefficient >|0.7|, one of the two variables was removed, and a final selection of five variables was used: mean diurnal temperature range (mean of the difference between monthly maximum and minimum temperatures [max temp - min temp]; bio2), maximum temperature of the warmest month (bio5), precipitation seasonality (coefficient of variation; bio15), precipitation during the wettest quarter (bio16), and precipitation during the coldest quarter (bio19).

Table 1. Full set of bioclimatic variables used for MaxEnt distribution models and PCA of climatic variables, for realized climatic niche analysis of *L. fulica*.

Bioclimatic variable	**Name**
bio1	annual mean temperature
bio2	mean diurnal range (Mean of monthly (max temp-min temp))
bio3	isothermality (bio2/bio7) (x100)
bio4	temperature seasonality (standard deviation x100)
bio5	max temperature of coldest month
bio6	min temperature of coldest month
bio7	temperature annual range (bio5-bio6)
bio8	mean temperature of wettest quarter
bio9	mean temperature of driest quarter
bio10	mean temperature of warmest quarter
bio11	mean temperature of coldest quarter
bio12	annual precipitation
bio13	precipitation of wettest month
bio14	precipitation of driest month
bio15	precipitation seasonality (coefficient of variation)
bio16	precipitation of wettest quarter
bio17	precipitation of driest quarter
bio18	precipitation of warmest quarter
bio19	precipitation of coldest quarter

Potential distribution models

Correlative species distribution models, such as maximum entropy (Maxent), statistically relate climatic variables directly to species occurrence (Dormann, Schymanski et al., 2012). The models used in this study were constructed using the Maxent 3.4.1 program (Phillips et al., 2008) and calibrated using 80% of the presence records. Linear and quadratic fits were also performed with the logistic output format, obtaining continuous values for the probability of environmental suitability ranging from 0 to 1. The area under the receiver operating characteristic curve (AUC) was used to evaluate model performance. AUC is a threshold-independent measure of a model's ability to discriminate presence from absence. It varies from 0.5 to 1.0; an AUC value of 0.5 shows that model predictions are not better than random, values <0.5 are worse than random, 0.5–0.7 signifies poor performance, 0.7–0.9 signifies reasonable or moderate performance, and >0.9 indicates high model performance. The significance of the climatic variables within the models was estimated using the contribution percentage of each variable (Elith et al., 2006).

We used seven models to evaluate the two hypotheses. To investigate whether the realized climatic niche of the snail has expanded, we tested whether differences existed between the climatic conditions of its native and current areas. For this purpose, we extrapolated the climatic conditions of the native area to the global climate (Model 1: native area to global projection) and projected the climatic conditions of the current distribution onto the global climate (Model 2: current to global projection).

As these models corroborated niche expansion, we then tested whether differences existed between the climatic conditions of the native area and Indo-Malayan + Australasian regions. We extrapolated the climatic conditions of the native area to the Indo-Malayan + Australasian region (Model 3: native to Indo-Malayan + Australasian projection) and the climatic conditions of the current Indo-Malayan + Australasian distribution to the Indo-Malayan + Australasian region (Model 4: current Indo-Malayan + Australasian to Indo-Malayan+Australasian projection). To test whether differences existed between the climatic conditions of the native area and Neotropics, we extrapolated the climatic conditions of the native area to the Neotropics (Model 5: native to Neotropics projection) and the climatic conditions of the current Neotropical distribution to the Neotropics (Model 6: current Neotropics to Neotropics projection). To test whether differences between Models 5 and 6 could be partially explained by the conditions of the Indo-Malayan + Australasian regions, we extrapolated the climatic conditions of the extended native distribution (native + Indo-Malayan + Australasian) to the Neotropical climatic conditions (Model 7: extended native to Neotropical projection).

All models were run for 500 iterations and 10 repetitions using bootstrapping as a resampling technique. The preparation analyses for the modelling (including format change, correlation analysis, layer cut, and value extraction) were performed with the raster (Hijmans, 2020), rgdal (Bivand et al., 2020), ascii (Hjage, 2020), vegan (Oksanen et al., 2019), letsR (Vilela & Villalobos, 2015), and devtools (Wickham et al., 2020) packages of RStudio 4.0.1.

Environmental PCA

To estimate potential changes in the realized climatic niche of the giant African snail among regions, we used PCA-env based on the framework proposed by Broennimann et al. (2012). First, we standardized the presence records through kernel density analysis using an environmental grid (1° × 1°) derived from the same five climatic variables as described in section 2.2. This analysis prevented biases owing to the spatial resolution of the variables using geographical and environmental spaces and corrected the density of the records according to the regional environmental space. We performed PCA-env for climatic conditions of the following pairs: native and global, native and Indo-Malayan + Australasian, native and Neotropical, and extended native (native + Indo-Malayan + Australasian) and Neotropical. We then used the PCA scores to calculate the niche overlap index (Schoener's D), which ranged from 0 (no overlap) to 1 (total overlap). These analyses were performed using the ecospat package (Di Cola et al., 2017).

The change in the realized climatic niche depicted by each PC1 × PC2 graph was described by niche expansion, stability, and "unfilling" (Guisan et al., 2014). Expansion, which occurs when a species colonizes new environments in the invaded areas, is estimated as the proportion of the non-native niche that does not overlap with the native niche. Stability, the tendency of a species to retain its niche in space and time, is estimated as the proportion of the non-native niche overlapping with the native niche. Unfilling occurs when the species only partially fills its niche in the invaded range and is estimated by the proportion of the native niche that does not overlap with the non-native niche.

Finally, we performed niche similarity and equivalence tests to evaluate the statistical significance of the observed overlap between native and non-native climatic conditions (Broennimann et al., 2012; Strubbe et al., 2015; Warren et al., 2008). The niche similarity test addresses whether the observed overlap between native and non-native niches is higher or lower than that between randomly shifted niches. The rejection of the similarity hypothesis indicates that the overlap of native and non-native climatic conditions is greater or smaller than expected. The niche equivalence test addresses whether the observed niche overlap is constant when randomly reallocating occurrences between two ranges. Rejection of the equivalence hypothesis indicates that the observed overlap between native and non-native niches is not constant. These analyses were performed using the ecospat package (Di Cola et al., 2017), and the similarity and equivalence tests were calculated with 1000 repetitions to generate the null distributions of D values in each comparison.

Results

Potential distribution models

Six of the seven potential distribution models obtained AUC values greater than 0.9, indicating high performance (for Model 2, AUC = 0.895, which is reasonable or moderate performance). In general, precipitation during the wettest (bio16) and coldest (bio19) quarters had the highest contribution. In the projections from the native area (Models 1, 3, and 5), precipitation during the coldest quarter (bio19) had the highest contribution. In the projections from the extended native area (Model 7) and the current distributions (Models 2 and 4), the precipitation during the wettest quarter (bio16) had the highest contribution. In the projection from the introduced range in the Neotropics (Model 6), the mean diurnal range (bio2) had the highest contribution, followed by similar percentages from bio16 and the maximum temperature of the warmest month (bio5) (Table 2).

Table 2. Values of the area under the curve (AUC) and percentage of contribution of the climatic variables in each of the potential distribution models with the MaxEnt algorithm for the giant African snail (*L. fulica*), with the highest percentages in bold. Climatic variables: mean diurnal range (bio2), maximum temperature of warmest month (bio5), precipitation seasonality (bio15), precipitation of wettest quarter (bio16), precipitation of coldest quarter (bio19).

Models	**AUC±SD**	**bio2**	**bio5**	**bio15**	**bio16**	**bio19**
1: native to global projection	0.935±0.012	17.2	1.4	8.5	26.9	**46**
2: current to global projection	0.895±0.001	17.5	10.9	0.7	**69.6**	1.4
3: native to indo-malayan+australasian projection	0.948±0.015	16.9	0.9	9.4	21.2	**51.7**
4: current indo-malayan+australasian to indo-malayan+australasian projection	0.922±0.002	5.9	5.5	2.7	**76.2**	9.7
5: native to neotropics projection	0.943±0.009	14.3	2.4	9.9	28.5	**44.8**
6: current neotropics to neotropics projection	0.906±0.007	**33.1**	**27.6**	3.5	**29.7**	6.1
7: extended native to neotropics projection	0.915±0.002	17.4	3	1.4	**65.6**	12.7

Global Models 1 and 2 showed a tropical distribution of *L. fulica*. The values and distribution of suitability differed among the Indo-Malayan, Australasian, and Neotropical regions (Figure 2A, B). The behavior of the climatic variables was also different in these models (see Supporting Information, Figure S1). Precipitation in the wettest quarter (bio16) was lower in Model 1 (0–3000 mm) than in Model 2 (0–4000 mm), and the suitability interval was narrower in Model 1 than in Model 2. Precipitation during the coldest quarter (bio19) had the same interval in both models (0–3000 mm); however, the suitability interval was narrower in Model 1 than in Model 2. In summary, the differences between the climatic conditions of the native and invaded areas suggest that the realized climatic niche of the snail has expanded.

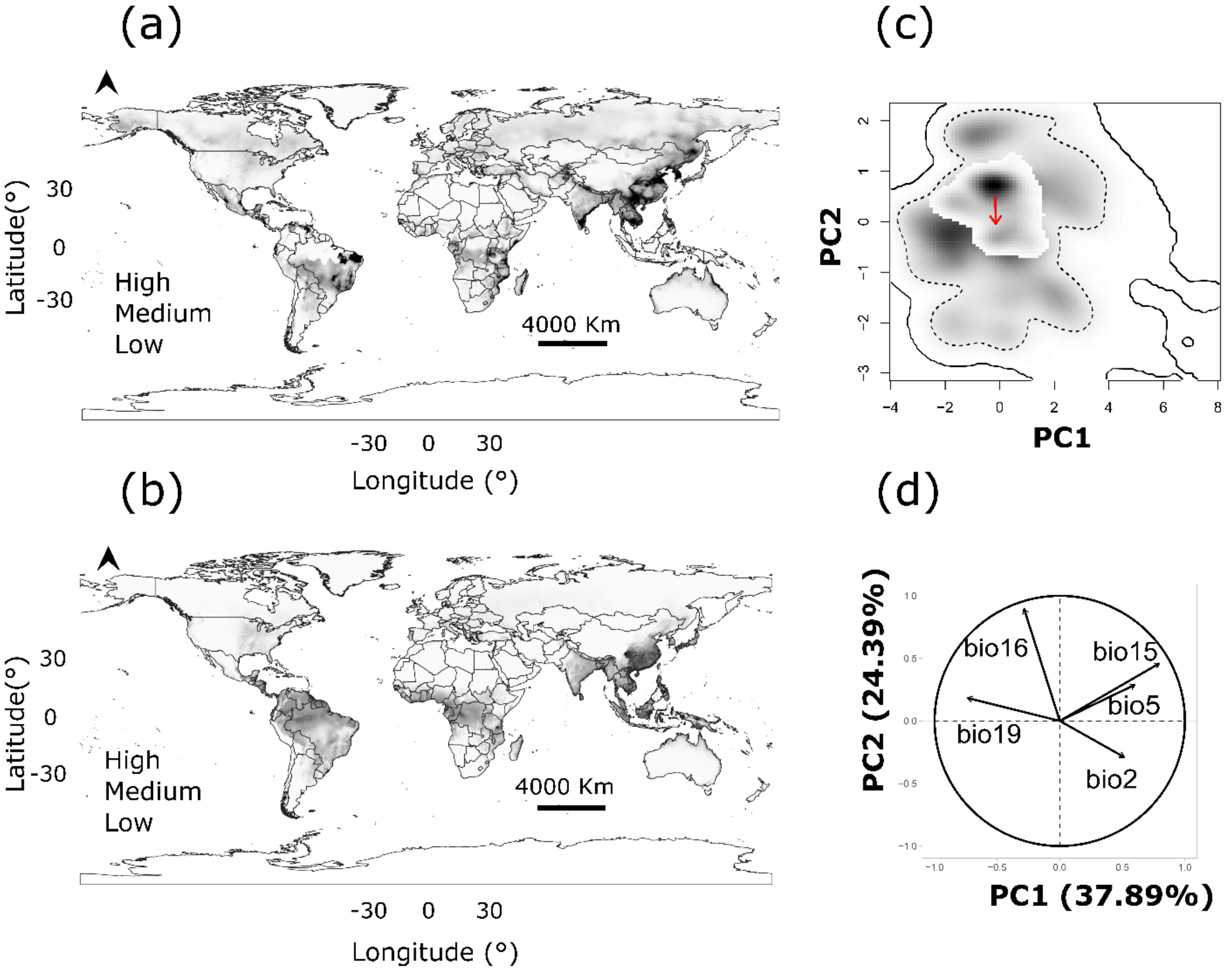

Figure 2. Models of the realized climatic niche of the giant African snail (*Lissachatina fulica)* in its native and non-native areas. Shading represents values of probability of environmental suitability, which were calculated using Maxent: high= 0.7–1, medium= 0.5–0.7, and low= 0–0.5. A. Potential distribution model projecting the native area of *L. fulica* into the entire current non-native area (Model 1: native to global projection). B. Potential distribution model with both the native and non-native areas of *L. fulica* (Model 2: current to global projection). C. Environmental principal component analysis (PCA-env) with the native climatic conditions and the current climatic conditions (native and global), illustrating the displacement of the centroid of the native climatic conditions toward the current climatic conditions (red arrow). D. Correlation circle of PCA-env showing the relationship between the climatic variables and the two PCA-env components.

The models of the Indo-Malayan and Australasian regions (Models 3 and 4) differed in suitability values observed in localities influenced by the Himalayas and southern India, as well as on the islands between the Indian and Pacific oceans (Figure 3A, B). The behaviors of the climatic variables in these models were different (see Figure S2). Precipitation during the wettest quarter (bio16) was smaller in Model 3 (0–3000 mm) than in Model 4 (0–4000 mm), and the suitability interval was narrower in Model 3 than in Model 4. Precipitation during the coldest quarter (bio19) was higher in Model 3 (0–3000 mm) than in Model 4 (0–2500 mm), whereas the suitability interval was narrower in Model 3 than in Model 4. Thus, differences were detected between the climatic conditions of the native area and the Indo-Malayan + Australasian regions.

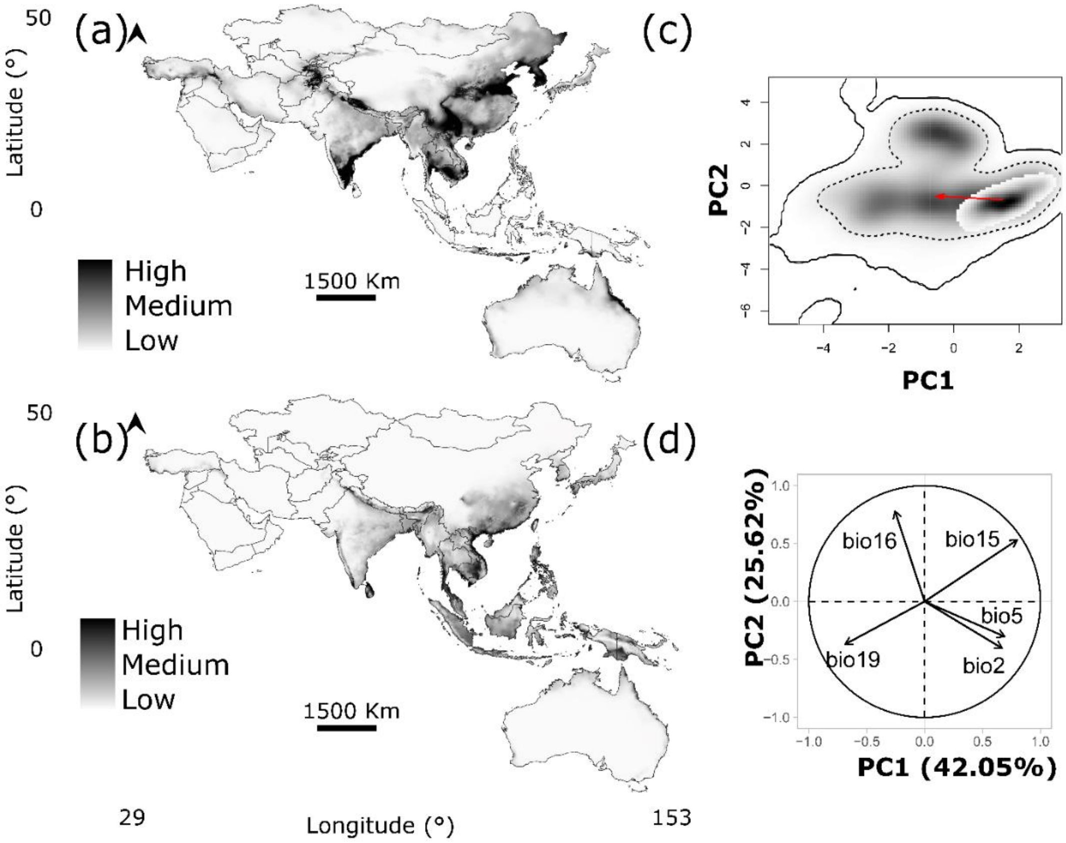

Figure 3. Models of the realized climatic niche of the giant African snail (*Lissachatina fulica*) in the Indo-Malayan and Australasian biogeographic regions. Shading represents values of probability of environmental suitability, which were calculated using Maxent: high= 0.7–1, medium= 0.5–0.7, and low= 0–0.5. A. Potential distribution model projecting the native area of *L. fulica* in the Indo-Malayan and Australasian regions (Model 3: native to indo-malayan+australasian projection). B. Potential distribution model in the Indo-Malayan and Australasian regions (Model 4: current indo-malayan+Australasian to indo-malayan+australasian projection). C. Environmental principal component analysis (PCA-env) with the native climatic conditions and the Indo-Malayan/Australasian climatic conditions (native and indo-

malayan/australasian), together with the displacement of the centroid (red arrow). D. PCA-env correlation circle showing the relationship between the climatic variables and the two PCA-env components.

The three models for the Neotropics (Models 5, 6, and 7) differed in their suitability values and in the localities where they predicted the presence of the giant African snail. Model 5 excluded a wide area in northern South America, specifically the Amazon, and localities influenced by the Andes (Figure 4A). In contrast, Model 7 (Figure 4B) had decreased suitability values, but the predicted localities with snails were similar to those predicted by Model 6 (Figure 4C). The behavior of the climatic variables in the Neotropics models (5, 6, and 7) also differed (see Figure S3). The mean diurnal range (bio2) intervals were slightly lower (4–22°C) for Model 5 than for Models 6 and 7 (2–22°C), and the suitability interval was narrower in Model 5 than in Models 6 and 7. For precipitation during the wettest quarter (bio16), the smallest interval occurred in Model 6 (0–2500 mm), followed by Model 5 (0–3000 mm) and Model 7 (0–5000 mm), and the suitability interval was narrower in Model 5 than in Models 6 and 7. For precipitation during the coldest quarter (bio19), Model 6 had the lowest interval (0–2000 mm), followed by Model 5 (0–3000 mm) and Model 7 (0–4000 mm), and the suitability interval was narrower in Model 5 than in Models 6 and 7. Hence, the differences between the climatic conditions of the native area and Neotropics could be attributable to Indo-Malayan and Australasian climate conditions.

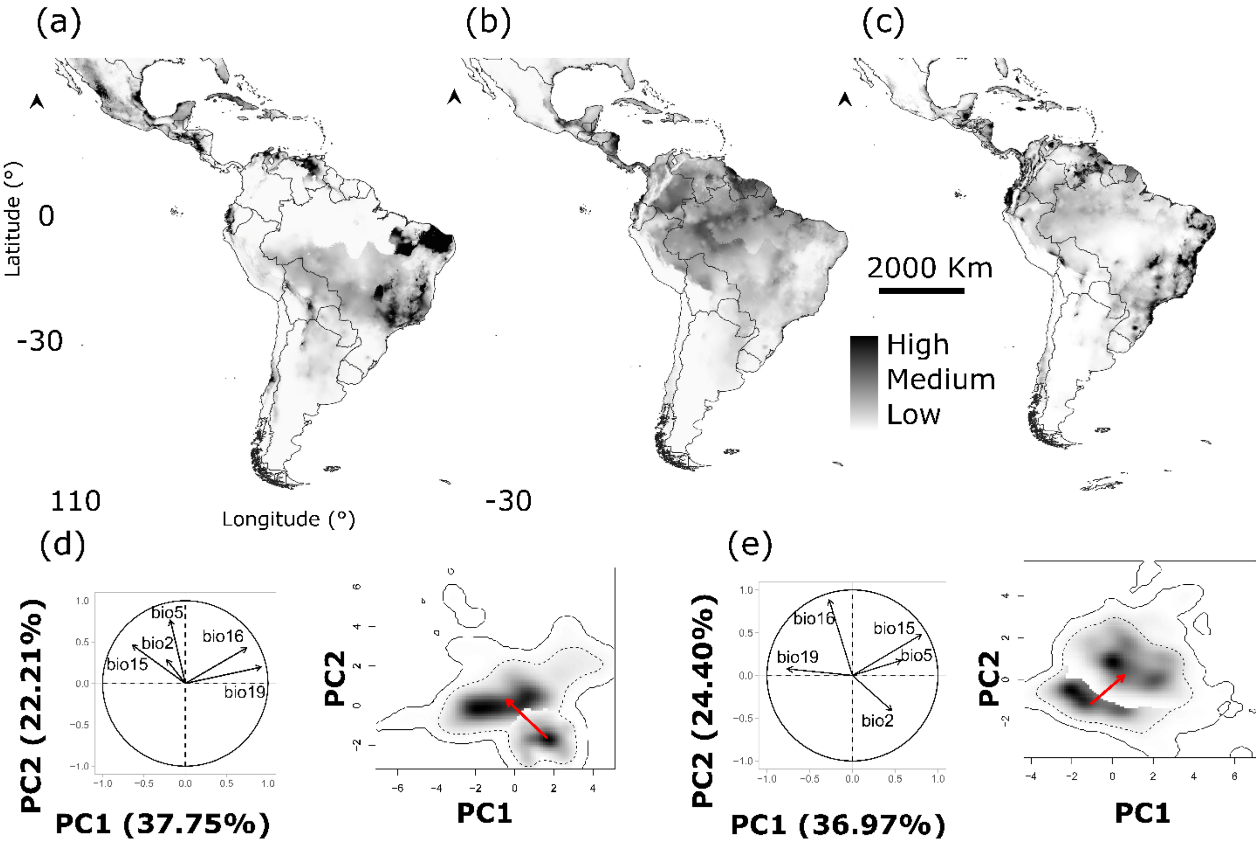

Figure 4. Models of the realized climatic niche of the giant African snail (*Lissachatina fulica*) in the Neotropical biogeographic region. Shading represents values of probability of environmental suitability, which were calculated using Maxent: high= 0.7–1, medium= 0.5–0.7, and low= 0–0.5. A. Potential distribution model projecting the native area of *L. fulica* in the Neotropical region (Model 5: native to neotropics projection). B. Potential distribution model projecting the native area and the Indo-Malayan and Australasian regions in the Neotropical region (Model 7: extended native to neotropics projection). C. Potential distribution model in the Neotropical region (Model 6: current neotropics to neotropics projection). D. Environmental principal component analysis (PCA-env) and the correlation circle with the native climatic conditions and the Neotropical conditions (native and neotropics). E. PCA-env and the correlation circle with native and the Indo-Malayan and Australasian climatic conditions, with the Neotropical conditions (extended native and neotropics). The red arrows indicate the displacement of the centroid of the native realized climatic niche toward the non-native realized climatic niche

Environmental PCA

In the PCA-env for native and global range, the first component (37.89%) was closely associated with precipitation during the coldest quarter (bio19), and the second component

(24.39%) was closely associated with precipitation during the wettest quarter (bio16). An expansion of the realized climatic niche of *L. fulica* was graphically observed (Figure 2C). Furthermore, the displacement of the centroid relative to the second component suggests that precipitation during the wettest quarter (bio16) influenced this change (Figure 2D). These results suggest that the realized climatic niche of the giant African snail has expanded with respect to precipitation conditions.

In the PCA-env for native and Indo-Malayan–Australasian parts of the range, the first component (42.05%) was equally associated with precipitation during the coldest quarter (bio19) and the two temperature variables (bio2 and bio5). The second component (25.62%) was associated more with precipitation during the wettest quarter (bio16) than with the other variables. Graphically, the climatic conditions where *L. fulica* occurs expanded from the native area toward the Indo-Malayan and Australasian regions, together with a displacement in the centroid that exceeded the limits of the native climate in the direction of the first component (Figure 3C, D). Thus, an expansion in climate conditions between native and Indo-Malayan and Australasian regions occurred and was mainly related to precipitation and temperature.

In the PCA-env for native and Neotropical parts of the range, and for the extended native and Neotropical areas, the first and second components explained ~37% and ~23% of the variation, respectively. In both PCA-env analyses, the first component was strongly associated with precipitation during the coldest quarter (bio19). The second component was strongly associated with the maximum temperature of the coldest month (bio5) in the native and Neotropical PCA-env and with precipitation during the wettest quarter (bio16) in the extended native and Neotropical PCA-env. Graphically, the centroid changed in both PCA-env analyses (Figure 4D, E). These results suggest that the changes that occurred between native and Neotropical climate conditions could be associated with Indo-Malayan–Australasian climate conditions.

In general, all PCA-env analyses showed an expansion of the realized climatic niche of *L. fulica* and low overlap (Schoener's *D* index; Table 3). The native and global PCA-env had an expansion of 0.60 and stability of 0.40. The PCA-env for native and global ranges and for native and Indo-Malayan–Australasian ranges showed no unfilling, indicating that all climatic combinations where *L. fulica* occurs in the native area were also occupied in the invaded areas. Unfilling values were only recorded in the PCA-env for the Neotropics, with the unfilling value of native and Neotropical areas being almost two times greater than that of the extended native and Neotropical areas (Table 3). Thus, these results suggest that the climatic conditions occupied by *L. fulica* in the Neotropics were better explained when the native, Indo-Malayan, and Australasian regions were included. For low overlap, the null hypotheses of similarity and equivalence tests were not rejected ($p > 0.05$), indicating that the observed overlap in all PCA-env is expected by chance and is constant.

Table 3. Percentage of niche expansion, stability, unfilling, and overlap index (Schoener's D) for each environmental PCA of *L. fulica.*

Comparison	**Expansion**	**Stability**	**Unfilling**	**D**
native and global	0.6038	0.3961	0	0.11
native and indo-malayan/australasian	0.7437	0.2562	0	0.06
native and neotropics	0.9763	0.0236	0.87	0.01
extended native and neotropics	0.1736	0.8263	0.47	0.15

Discussion

According to the Maxent models used in this study, the realized climatic niche of *Lissachatina fulica* has expanded and varies geographically among non-native areas. PCA-env analyses also showed climatic expansion between the native and invaded areas. These observed changes in climatic niche add to the knowledge base of invasive species niche dynamics, in which niche shifts, niche conservatism, or environmental nonequilibrium could be observed in native and non-native parts of ranges (e.g., Pili et al., 2020; Strubbe et al., 2013). The expansion of the realized climatic niche of *L. fulica*, as suggested by our results, could be associated with its spread (Africa to Indo-Malaya and Australasia to Neotropics). Species with wide ecological tolerances, when transported to other areas, might be able to spread either because invaded regions are climatically similar to their native range (Wiens et al., 2010) or because of increased ecological opportunities in regions that are climatically different (Alexander & Edwards, 2010). Furthermore, the intentional introductions along the Indo-Malayan and Australasian regions, and the late introduction in the Neotropics, could benefit the establishment of *L. fulica* not only in areas that are climatically similar but also in areas that are climatically different from its native area (Liu et al., 2020a). Therefore, the actual realized climatic niche of *L. fulica* could be explained by its wide adaptive potential, release of constraints, introduction type, and introduction time.

The expansion of the realized climatic niche of the giant African snail could be facilitated by its high adaptive potential associated with the release of dispersal barriers (Alexander & Edwards, 2010; Broennimann et al., 2007; Raut & Barker, 2002). Populations of *L. fulica* introduced from the species' native area into Indo-Malayan and Australasian regions were found in areas with the same precipitation and temperature regime. Without constraints, and owing to their high fecundity (e.g., ~400 eggs per snail; Roda et al., 2016), these snails can rapidly establish populations in conditions climatically similar to their native area. However, *L. fulica* also occupies areas with different precipitation and temperature regimes because of its wide physiological tolerance to variations in temperature and relative humidity (Sharma & Dickens, 2018) and strategies such as estivation (Rahman & Raut, 2010). In summary, the ability to survive and reproduce under a wide array of conditions, together with the release of constraints, could have supported the expansion of *L. fulica* over the Indo-Malayan and Australasian regions.

The introduction of the giant African snail was intentional (Raut & Barker, 2002; Thiengo et al., 2007). Since the first intentional introduction of the Indo-Malayan in Calcutta, located in the western region of India, this species has invaded semi-urban, peri-urban, and rural areas (Sarma et al., 2015). When introduced into urban or disturbed environments, invasive species can experience selection pressures that may preadapt them for establishment in other locations (Borden & Flory, 2021). Therefore, intentional introductions allowed the mollusc to overcome difficulties related to each stage of invasion in disturbed environments (Lake & O'Dowd, 1991; Thiengo et al., 2007), resulting in the colonization of the Australasian islands and the Neotropics. The intentional introductions could explain the stability and the low nonsignificant overlap because the increase in introduction events could provide more opportunities for the occupation of native climatic conditions. Thus, intentional introduction and subsequent uncontrolled expansion could have facilitated all invasion stages of *L. fulica,* resulting in this actual distribution.

The long invasion process gave shape to the realized climatic niche of *L. fulica*. By using correlative models, Liu et al. (2020a) found that the year of introduction was positively associated with niche breadth ratio but negatively associated with niche similarity. Correlative models require environmental equilibrium and technically good transferability. As environmental equilibrium presupposes that the species occurs throughout most suitable environmental conditions (Dormann, Schymanski et al., 2012), equilibrium absence suggests less residence time; in other words, recently introduced species have not yet dispersed to occupy all potential favorable conditions they can survive (Liu et al., 2020a). Model transferability is the performance of the native-range model that was extrapolated to correctly predict occurrences in the introduced range. Transferability is higher in species introduced more recently; however, this can vary among continents (Liu et al., 2020b). Therefore, the consideration of native conditions and the chronology of continental introductions were important for investigating whether the expansion of the realized climatic niche of *L. fulica* was influenced by the spread from its native to the non-native areas.

The difference in the percentage of unfilled niches between the Indo-Malayan + Australasian and Neotropical regions could be explained by the time of introduction (Liu et al., 2020a). The expansion values of 74% in the Indo-Malayan and Australasian regions and 17% in the Neotropics indicate that this species has a high dispersal and establishment capacity. Its dispersal can be explained by historical and commercial events at regional and local spatial scales, on which an association with land transport routes has been proposed (Adhikari et al., 2020). In Neotropical localities, the establishment of *L. fulica* was highly favored by urban environments (Thiengo et al., 2007), which could have contributed to the 17% expansion in less than half a century. Owing to the differences in the time of establishment in different populations, investigating differences in ecological attributes such as density, age structure, and maturation times among biogeographic regions would be interesting.

For the Neotropical region, the differences between the Maxent models as well as the expansion and unfilling values could also be explained by the time of introduction and establishment of the populations. Although the first introductions occurred at the end of the 20th century (Thiengo et al., 2007), dispersal and establishment were identified only in the first decade of the 21st century (Vogler et al., 2013). For these reasons, the Maxent models constructed by

Borrero et al. (2009) and Vogler et al. (2013), although identifying a large part of climatically suitable areas, did not establish the species in the entire region. The climatically suitable areas described in Borrero et al. (2009) coincided considerably with the areas predicted by Model 5; those described in Vogler et al. (2013) coincided substantially with Models 6 and 7. The current Neotropical distribution of *L. fulica* is better explained by Models 6 and 7, indicating that the species is established in localities with native and Indo-Malayan climatic conditions. Additionally, half of the unfilling in the PCA-env of the extended native and Neotropical areas compared to the unfilling observed in the PCA-env of native and Neotropical areas could be due to the existence of potential localities without establishment. Thus, what has been observed in the Neotropical region can provide information on the establishment potential of this species in regions such as the Nearctic (Olson et al., 2001) and even under conditions of climate change (Sarma et al., 2015).

In conclusion, the spread of *L. fulica* is correlated with an expansion of its realized climatic niche. The expansion could occur because of its first introduction in areas climatically similar to its native area and urban environments. Subsequently, the regional and local dispersal associated with historical and commercial events and land transportation routes could contribute to the establishment of this species, which was preadapted to urban environments, more easily in climates different from those of its native area. Thus, the climatic niche of this invasive mollusc is the result of its native, Indo-Malayan, Australasian, and partly Neotropical conditions; and for Neotropical conditions, the short time since initial introduction is responsible for the niche expansion value of only 17% and niche unfilling value of 47%. Finally, with this analysis, we hope to encourage further ecological studies of giant African snail populations in regions where they are established to better understand how this species can establish itself in non-tropical regions or how it may expand its invasive potential under different future climate scenarios.

Acknowlegments.

We thank the Coordenação de Aperfeiçoamento de Pessoal de Educação Superior (CAPES, CODE 001) for the doctoral fellowship awarded to the first author, and Rodrigo Lozano for support with the construction of the analysis corresponding to the MaxEnt models.

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CAPITULO 3. Variation in the population density of the giant African snail (*Lissachatina fulica*) in the Neotropical region

Paper published in Caldasia accepted in December 13 2021

Available online January 06 2022

Patiño-Montoya, A., Giraldo, A., & Tidon, R. (2022). Variation in the population density of the giant African snail (*Lissachatina fulica*) in the Neotropical region. *Caldasia*, *44*(3), 627-635.

Cited by:

Teles, W. S., Silva, D. D. P., Vilela, B., Lima-Junior, D. P., Pires-Oliveira, J. C., & Miranda, M. S. (2022). How Will the Distributions of Native and Invasive Species Be Affected by Climate Change? Insights from Giant South American Land Snails. *Diversity*, *14*(6), 467.

Abstract.

The giant African snail (*Lissachatina fulica*), one of the 100 world's worst invasive species, has been recorded in the Neotropical region since the 1980s. Temperature and precipitation variables affect snail population density; however, as far as we know, these relationships have not been investigated for *L. fulica* on a regional scale. Here, we made the first description of variation in population density of *L. fulica* in the Neotropical region using a literature search, descriptive statistics, and a Principal Component Analysis (PCA). We found 22 studies covering 36 localities in six countries. The mean snail density was 11.55 ± 28.32 ind/m^2, with the lowest value recorded in Cuba (0.0002 ind/m^2) and the highest value recorded in Venezuela (150 ind/m^2). These values were recorded between 21% to 93% of Human Footprint, 710 mm to 4438 mm of Annual Precipitation, 13 ºC to 27 ºC, Mean Temperature of the Coldest Quarter, and 3 ºC to 40 ºC of Temperature Seasonality. The PCA suggested that low densities can occur in various environmental conditions, whereas medium and high densities seem to appear in more specific climatic combinations. In conclusion, increased densities of *Lissachatina fulica* in the Neotropics seem to be influenced by climatic variations, especially the Mean Temperature of the Coldest Quarter and Annual Precipitation, supporting previous finding in the literature regarding the snail stablishment. Future monitoring of this invasive species, performed at expanded spatial and temporal scales, may provide tools to establish a relationship between snail density values and impact.

Keywords**.** *Achatina fulica*, Invasive snail, Invasion Biology, Population ecology.

Introduction

The giant African snail, *Lissachatina fulica* (Bowdich 1822), is one of the 100 world's worst invasive species (Lowe *et al.* 2004, Thiengo *et al.* 2007). In the invaded areas, *L. fulica* can cause impacts on health, agriculture, and the economy. In health, the snail can act as an intermediate host for nematode species of the genera *Angiostrongylus* and *Aelurostrongylus*, which cause eosinophilic meningitis and abdominal angiostrongyliasis in humans, besides other diseases in domestic animals (Fischer *et al.* 2010). *L. fulica* is recognized as a generalist herbivore that feeds on various cultivated plant species (Thiengo *et al.* 2007). Finally, the presence of *L. fulica* entails an expense of the public herald for the potential consequences and control that must be carried out (Roda *et al.* 2018). Therefore, ecological research on *L. fulica* is necessary to mitigate the negative impacts of its presence through the construction of comprehensive management plans.

In the Neotropics, *L. fulica* was first recorded in the 1980s in Martinique and Brazil (Mead and Palaci 1992, Santana-Teles *et al.* 1997). By the beginning of the 21st century, it has already been recorded in Cuba (Vázquez and Sanchez 2014), Venezuela (Martínez-Escarbassiere *et al.* 2008), Colombia (De la Ossa-Lacayo *et al.* 2012), Ecuador (Goldyn *et al.* 2017) and Argentina (Gutierrez-Gregoric *et al.* 2011). After recording *L. fulica*, some countries have established regulations to control and manage the snail, besides inciting research to provide baseline knowledge (MAVDT 2011). However, this control has been based on the manual collection and subsequent culling with chemical or physical means (Thiengo *et al.* 2007), which requires ecological knowledge of local populations. As the species is widely distributed in the Neotropics (Darrigran *et al.* 2020), a better understanding of the mollusk population dynamics in this region could help for enhancing control and management plans.

Density is one of the population parameters taken into account to assess whether an exotic population is established and the possible impact it produces. In *L. fulica*, it is postulated that a population density higher than 10 ind/m^2 is already a cause for concern as an established population (De la Ossa *et al.* 2017). This argument is based on the fact that population density influences parameters related to fitness such as growth, fecundity, egg viability, and dispersal (Dickens *et al.* 2018). Nevertheless, the relationship between population density and the impact of an invasive population is not linear (Jackson *et al.* 2015) and can be affected by other ecological factors such as the trophic level (Bradley *et al.* 2019). We would also expect that density is also affected by temperature and precipitation; however, as far as we know, these relationships have not been investigated for *L. fulica* on a regional scale. In heterogeneous environments, such as those found in the Neotropics, understanding the relationships between density and climate should contribute to modeling the link between density and impact.

Here, we estimated the amplitude of climatic and anthropic variables where the population density of *L. fulica* was recorded in the Neotropical region. Through the association of density values to climatic and anthropic variables, we aim to answer the following questions: How much does the population density of *L. fulica* varies in the Neotropical region? Which are the main climatic variables affecting the density of *L. fulica* in the Neotropical Region? How do these climatic variables, as well as the human footprint, affect snail density? This study is the first descriptive approach on the variation of the population density of *L. fulica* in the Neotropics, focusing on the intervals of climate and anthropic intervention where the species was recorded. We expect that this work will provide subsidies for decision-making for managing the giant African snail in the countries of the region.

Materials and Methods**.**

A directional search with the keyword "*Achatina fulica*" was performed in Google Scholar in English, Spanish and Portuguese. This keyword corresponds to the old name for the species, as the genus change from *Achatina* to *Lissachatina* was recently accepted (https://www.marinespecies.org/aphia.php?p=taxdetails&id=881469). We downloaded all documents presenting numerical values of population density in neotropical countries. Because some documents did not show the exact collection coordinates, the geographic coordinates of the localities recorded in each document were approximated in Google Earth, using the center of the locality recorded. To access the anthropic intervention in the Neotropics, we used the global human footprint index, which is expressed as a percentage representing the relative human influence in each terrestrial biome (WCS 2005). To access environmental parameters in the Neotropics, we used the nineteen current climate variables from WORLDCLIM 2.1 (https://www.worldclim.org/data/worldclim21.html, resolution 2.5 arcminutes). We extracted the Human Footprint and climate values for locations with density records with the vegan (Oksanen *et al.* 2020) and raster (Hijmans 2021) packages in RStudio 4.1.0 (Supplementary material 1, Table S1).

Based on (Albuquerque *et al.* 2009, Vogler *et al.* 2013), we identified Human Footprint, Annual Precipitation, Mean Temperature of the Coldest Quarter, and Temperature Seasonality as the most relevant predictors for *L. fulica* establishment. Then, we categorized each of these variables as "low" and "high", taking as reference their median value in the collection sites, and constructed boxplots using density values of each point as the response variable.

To explore the population density variation of *L. fulica* in the Neotropical region, we performed a principal component analysis (PCA). Variation was represented in a Cartesian space defined by the human footprint and the 19 climatic variables. Density values were classified as low (<0.001 to 3.35 ind/m^2), medium (4.03 to 9.2 ind/m^2), and high (10.45 to 150 ind/m^2), taking into account the median of the data and the suggestion of 10 ind/m^2 in the study of De la Ossa *et al.* (2017). The PCA and the graphical representation were elaborated with the packages FactoMineR (Lee *et al*. 2008) and factoextra (Kassambara and Mundt 2020) in RStudio 4.1.0.

Results

In total, we found 22 papers estimating the population density of *L. fulica* in the Neotropics. These papers record snail density in 36 localities covering six countries: Argentina, Brazil, Colombia, Ecuador, Venezuela, and Cuba, from 2004 to 2020 (Fig. 1a). For these records, the mean density was 11.55 ± 28.32 ind/m^2 and the median was 4.03 ind/m^2. The lowest and highest densities were reported in Cuba (Havana, 0.0002 ind/m^2) and Venezuela (Andres Bello, 150 ind/m^2). In most sites (80%) density values scored below 10 ind/m^2 (Table 1).

Table 1. Population density values at localities in the Neotropical region.

Reference	**Country**	**Locality**	**Ind/m^2**
Gutiérrez-Gregoric *et al.* 2011	Argentina	Puerto Iguazú	107.6
Gutiérrez-Gregoric *et al.* 2013		Corrientes	28
Santana and Batalla 2018	Brazil	Caraguatatuba, SP	0.186
de Almeida 2018		Santo Antonio de Padua, RJ	0.23
Oliveira *et al.* 2013		Santana, Amapá	6.15
Albuquerque *et al.* 2008		Lauro Freitas, Bahia	4.03
Albuquerque *et al*. 2009		Lauro Freitas, Bahia	2.2
Fischer *et al.* 2010		Paranagua	1.9
Lima and Guilherme 2018		Rio Branco, Acre	1.86
Simião and Fisher 2004		Pontal do Paraná	0.1
Miranda *et al.* 2015		São Vicente, SP	0.07
Cano 2018	Colombia	Natagaima, Tolima	18.67
Cano 2018		Lerida, Tolima	17.33
Cano 2018		Alvarado, Tolima	12.67
Cano 2018		Chaparral, Tolima	11.83
Cano 2018		Honda, Tolima	11.2
Cano 2018		Venadillo, Tolima	9.2
Cano 2018		Mariquita, Tolima	8.86
Cano 2018		Carme de Apicalá, Tolima	8
Cano 2018		Purificacion, Tolima	7.33
Cano 2018		Armero Guayabal, Tolima	6.4
Cano 2018		Ibague, Tolima	4.89
Avendaño and Linares 2015		Cundinamarca	4.6
Cano 2018		Melgar	4.34
Giraldo *et al.* 2017		Valle del Cauca	5.9
Avendaño and Linares 2015		Valle del Cauca	2
Avendaño and Linares 2015		Boyacá	1.8
Avendaño and Linares 2015		Meta	1.3
Avendaño and Linares 2015		Norte de Santander	1.1
De la Ossa-Lacayo *et al.* 2017		Tolú, Sucre	0.21
De la Ossa-Lacayo *et al.* 2017		Corozal, Sucre	0.09

De la Ossa-Lacayo *et al.* 2017		Sincelejo, Sucre	0.03
De la Ossa *et al.* 2017		Sincelejo, Sucre	0.004
De la Ossa-Lacayo and De la Ossa 2014		Sincelejo, Sucre	0.004
De la Ossa-Lacayo and De la Ossa 2014		Sampues, Sucre	0.003
Villavicencio-Abril *et al.* 2020	Ecuador	Sto Domingo de los Tsáchilas	3.35
Cuasapaz-Sarabia and Salas 2019		Guayas	10.45
Goldyn *et al.* 2017		Puyo	8.4
Herrera *et al*. 2016	Venezuela	Andres Bello	150
Vazquez and Sanchez 2014	Cuba	Havana	<0.001

Regarding the relevant predictors for *L. fulica* establishment suggested by the literature, we found density records in the following ranges: Human Footprint from 21% to 93%, Annual Precipitation between 710 mm to 4438 mm, Mean Temperature of the Coldest Quarter between 13 ºC to 27 ºC, and Seasonality of Temperature between 3 ºC to 40 ºC. In general, we found no density pattern between low or high values of these variables (Fig. 1b).

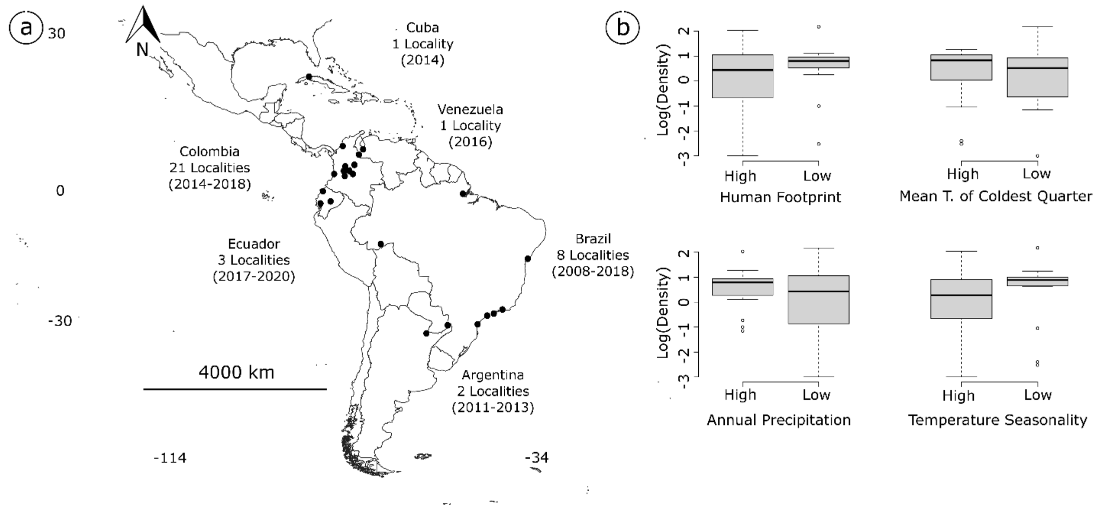

Figure 1. Density of *Lissachatina fulica* in the Neotropical Region. a. Map showing the sites where density data were recorded. b. Density variation in sites under high and low values of Human Footprint, Annual Precipitation, Mean Temperature of the Coldest Quarter and Temperature Seasonality; the categorization of the environmental variables into high and low was based on their median.

The first two components of the PCA explained 63.5% of the variance of the environmental data (Supplementary material 2, Table S2). Mean Temperature of the Coldest Quarter (bio11) and Mean Temperature of the Driest Quarter (bio9) had the largest contributions to the first component. In contrast, Annual Precipitation (bio12) and Precipitation of the Wettest Month (bio13) had the largest contributions to the second component. Human Footprint was the least contributing variable for both components (Supplementary material 2, Table S3). The Cartesian space defined by PC1 and PC2 shows that sites presenting low snail densities are distributed across the two components, indicating that different climatic combinations can maintain *L. fulica* populations. On the other hand, most sites presenting medium snail densities show positive values of the first component, whereas most sites presenting high densities show negative values of the second component. This pattern suggests that density increase can be influenced by specific temperature and precipitation ranges (Figure 2).

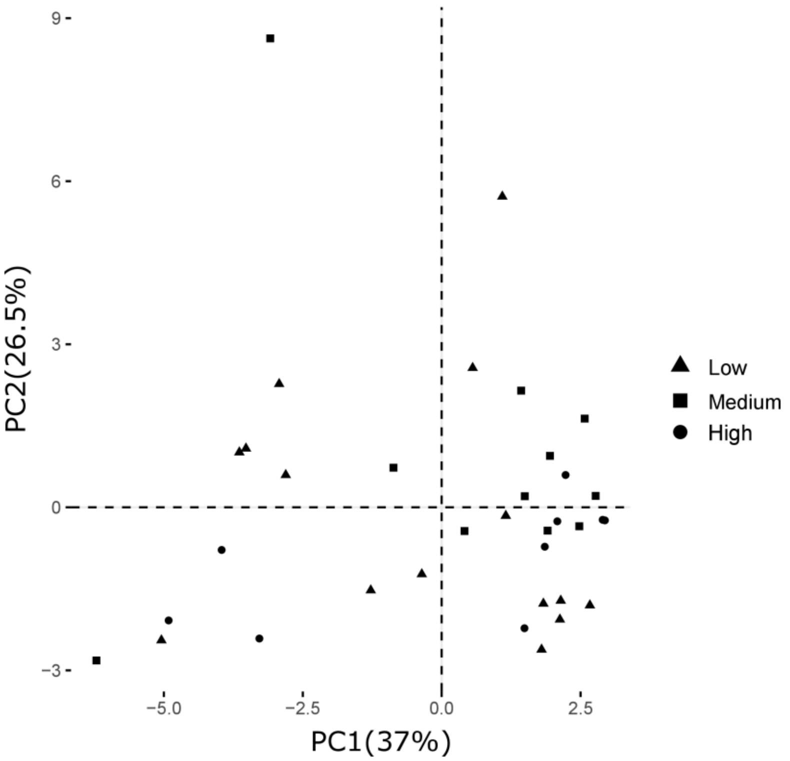

Figure 2. Principal Component Analysis (PCA) with the values of Human Footprint and climatic variables of the localities with recorded population density in the Neotropical region. Each point represents a locality, and its shape represents the recorded density (Triangle <0.001 to 3.35 ind/m^2, Square 4.03 to 9.2 ind/m^2, Circle 10.45 to 150 ind/m^2).

Discussion

In this study, we found a high variation in the population density of *L. fulica* in six Neotropical countries. Low densities of the species can be maintained in an array of environmental conditions, but density increase seems to be influenced by the Mean Temperature of the Coldest Quarter (bio11) and Annual Precipitation (bio12). The possible influence of bio11 on *L. fulica* density seems to be associated with establishment (Vogler *et al.* 2013) and survival (Sharma and Dickens 2018). Annual Precipitation, in turn, may be related to physiological processes, like estivation (Rahman and Raut 2010), that influence population dynamics. However, the low number of localities with density records does not capture all the environmental conditions that could explain the variation in density of the invasive mollusk.

Our data did not show clear effects of human intervention on snail population density. The human footprint did not contribute significantly for the principal components, and the highest population density (150 ind/m^2) was recorded in a site presenting the lowest value of human footprint (21%) (Supplementary material 1). In general, disturbed areas provide unique habitat opportunities and potential refugia for invasive alien species (Cadotte *et al.* 2017) and may even promote adaptation (Borden and Flory 2021). What is interesting in the case of *L. fulica* is its ability to maintain populations in low percentages of the human footprint, which would turn conservation units and rural areas of countries into refuges for the species (Fischer *et al.* 2010). Since *L. fulica* can occur from low to high human intervention, we call attention to direct management strategies in rural human populations.

According to the latest distribution model of the species in South America (Vogler *et al.* 2013), localities with low temperatures and high seasonal variation are less suitable for *L. fulica*. However, in our study the highest density values (150 - 107.6 ind/m^2) were found in localities with 4 °C and 34 °C temperature seasonality (Supplementary material 1). It is, therefore, possible to postulate that after establishment, *L. fulica* can maintain high densities in high seasonality. In Andrés Bello, a tropical locality presenting a very weak seasonal climate, the high snail density could be explained by resource availability (Martínez-Escarbassiere *et al.* 2008, Herrera *et al.* 2016). In the subtropical locality Puerto Iguazú, the high density could be explained by the sampling period: the collections were conducted in March, when temperatures around 31 °C and humidity around 70% (Gutiérrez-Gregoric *et al.* 2011) represent optimal conditions for *L. fulica*.

This difference demonstrates that temporal monitoring is necessary to identify the influence of climate and resource availability on the viability of populations.

In conclusion, the population density of *Lissachatina fulica* in the Neotropical region would be more influenced by climatic variables than by the degree of anthropogenic intervention. In this study, we postulate the Mean Temperature of the Coldest Quarter (bio11) and Annual Precipitation (bio12) as key climate variables influencing snail density, supporting the results found by Albuquerque *et al.* (2009) and Vogler *et al.* (2013) regarding *L. fulica* establishment. We call attention to the ability of the species to maintain low population densities over wide ranges of environmental variables. Since at high population densities, there is a greater perception of damage (Jackson *et al.* 2015), low-density localities are ignored when establishing control actions. In the future, a spatial and temporal expansion of local monitoring of this invasive species may provide sufficient tools to develop a relationship between density values and the impact produced by the species in the region.

Participation of authors.

APM Conceived ideas, data analysis and writing; AG data analysis and writing; RT data analysis and writing.

Acknowledgments.

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Code 001.

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CAPITULO 4. Caracterização de uma população não nativa de *Lissachatina fulica* e sua resposta à variação climática e presença de recursos

Resumo.

O caracol gigante africano *Lissachatina fulica*, um gastrópode invasor, é reconhecido por sua tolerância climática e associação com ambientes urbanos. Assumindo que a resposta de espécies invasoras ao clima e presença de recursos é contexto-específica, este trabalho caracteriza uma população não nativa de *L. fulica*, associa sua densidade populacional, porcentagens de indivíduos mortos, indivíduos com ovos, e hermafroditas, e avalia sua resposta à pequenas variações climáticas e presença de recursos. Foram realizadas 25 campanhas de coleta de *L. fulica* no Distrito Federal, nas estações chuvosas de 2020-2021 e 2021-2022. Mediante correlações foram associadas par a par a densidade populacional, porcentagem de indivíduos mortos, porcentagem de indivíduos com ovos e porcentagem de indivíduos hermafroditas. Com um modelo linear se relacionou a porcentagem de indivíduos com ovos com a densidade populacional, porcentagem de indivíduos mortos e porcentagem de indivíduos hermafroditas. A resposta a pequenas variações climáticas foi avaliada mediante correlações da densidade populacional, porcentagens de indivíduos mortos, indivíduos com ovos, e hermafroditas com temperatura máxima, umidade relativa mínima e precipitação de 8, 15 e 30 dias anteriores a cada coleta. Com modelos lineares se avaliou a resposta da porcentagem de indivíduos mortos e a porcentagem de indivíduos com ovos com a temperatura máxima, umidade relativa mínima e a precipitação de 30 dias. A resposta aos recursos se avaliou mediante modelos lineares entre a densidade populacional, porcentagens de indivíduos mortos, indivíduos com ovos, e hermafroditas com recursos. A porcentagem de indivíduos com ovos está associada positivamente com a porcentagem de indivíduos hermafroditas, e negativamente com a porcentagem de indivíduos mortos e densidade populacional. A porcentagem de indivíduos mortos e a porcentagem de indivíduos com ovos respondem com variações climáticas. A densidade populacional e a porcentagem de indivíduos hermafroditas respondem com presença de recursos. Em conclusão, a resposta da população não nativa de *L. fulica* é complexa, pois cada atributo responderia de forma diferente às variações climáticas e presença recursos, regulando os outros indiretamente.

Palavras chave. *Achatina fulica*, Caracol gigante africano, ecologia populacional, variação climática, disponibilidade de recursos.

Introdução.

O sucesso no estabelecimento de uma espécie invasora depende em parte da sua capacidade de adaptação na área não nativa depois da introdução (Duncan & Williams, 2002; Moran & Alexander, 2014; Enders et al., 2020). A adaptação ao clima da área não nativa vai depender em parte do nicho climático realizado da espécie antes da introdução (Wiens et al., 2010). Além do clima, a adaptação na área nativa é determinada pela heterogeneidade ambiental e a presença de recursos nestes locais (Sher & Hyatt, 1999; Melbourne et al., 2007; Enders et al., 2020). Portanto, o sucesso das espécies invasoras em locais não nativos depende das condições especificas de clima e recursos que serão encontradas na área invadida.

O clima pode regular populações invasoras, já que elas provavelmente se estabelecem primeiro em climas similares à área nativa (Wiens et al., 2010). No entanto, a adaptação ao clima da área não nativa varia entre as espécies invasoras. Em alguns casos, populações invasoras estão predispostas a uma ampla gama de condições climáticas, permitindo a colonização de diferentes ambientes (Querns et al., 2022). Em outros, desenvolvem tolerância à temperatura em indivíduos adultos (McCann et al., 2018) mas não em jovens (Wijethunga et al., 2022). Assim, a resposta de uma espécie invasora ao clima dependerá de como seus atributos de história de vida responderão em cada população não nativa.

Distúrbios antrópicos podem disponibilizar recursos que promovem o estabelecimento de espécies invasoras (Meyer et al., 2021). As cidades, por exemplo, facilitam o transporte e estabelecimento dessas espécies (Borden & Flory, 2021) porque oferecem uma série de oportunidades ecológicas, como maior pressão de propágulo, menos interações negativas, presença de refúgio e de alimento (Cadotte et al., 2017; Borden & Flory, 2021). Porém, quando se investiga a associação entre a presença de espécies invasoras e urbanização, a conclusão dependerá de como a invasão e a urbanização são medidas (Gippet et al., 2022). Portanto, a presença de recursos nas cidades influencia as espécies invasoras, mas, a resposta da espécie invasora pode ser contexto-especifica.

O caracol gigante africano (*Lissachatina fulica*) é um gastrópode listado dentre as 100 piores espécies invasoras do mundo (Lowe et al., 2000). As populações não nativas apresentam uma ampla variação na densidade populacional, bem como em características morfométricas e reprodutivas. Na densidade populacional é possível observar populações com densidades de <1 ind/m^2 (Vázquez & Sanchez, 2014) até >100 ind/m^2 (Gutierrez-Gregoric et al., 2011). O tamanho máximo dos indivíduos varia de ~60mm até 200mm (Barçante, 2005), indivíduos grávidos podem variar entre ~35mm até ~60mm, e o número máximo de ovos por individuo atinge 460 ovos (Roda et al., 2016). Tendo em vista que as características anteriores são influenciadas pela densidade populacional em laboratório (Dickens et al., 2018a; 2018b) e em campo (Raut & Ghara, 1989), a resposta de *L. fulica* em uma localidade não nativa deve ser avaliada holisticamente.

O caracol gigante africano apresenta uma ampla tolerância climática. Ele sobrevive de 8°C a 38°C e se reproduz de 22°C a 32°C (Sharma & Dickens, 2018). As atividades de alimentação e reprodução de *L. fulica* estão sincronizas com os meses de alta precipitação (Albuquerque et al., 2009; Valerio & Ferreira, 2018; Silva et al., 2019, 2022). Nos meses mais secos, a baixa umidade relativa desencadeia o processo fisiológico de estivação (Rahman & Raut, 2010). Por isso, alguns modelos climáticos preveem um baixo risco de estabelecimento de *L. fulica* em regiões com altas temperaturas e baixa precipitação (Sarma et al., 2015). Assim, o clima desempenha um papel importante nas populações de *L. fulica*.

O caracol gigante africano tem sido associado a ambientes urbanos (Albuquerque et al., 2008; Miranda et al., 2015; Goldyn et al., 2016), onde ocorre principalmente em locais que fornecem abrigo e alimento, como lotes abandonados, jardins e quintais (Thiengo et al., 2007; Albuquerque et al., 2008). Para caracterizar o uso das áreas urbanas pela espécie, a abundância de *L. fulica* tem sido estimada tanto em gradientes de perturbação (Lake & ODowd, 1991) como em categorias qualitativas de habitat urbano (Albuquerque et al., 2008, 2009). A resposta dos atributos reprodutivos a ambientes urbanos, por outro lado, ainda não foi explorada.

Assumindo que a resposta de espécies invasoras ao clima e presença de recursos é contexto-específica, neste trabalho caracterizamos a população não nativa de *L. fulica* localizada no Distrito Federal (DF). Além disso, como a densidade populacional pode influenciar características morfométricas e reprodutivas, investigamos a associação entre densidade populacional, porcentagem de indivíduos mortos, porcentagem de indivíduos com ovos, e porcentagem de indivíduos hermafroditas. Por último, avaliamos como a população de *L. fulica* no DF responde a pequenas variações no clima e na presença de recursos. A resposta de *L. fulica* foi inferida pela densidade populacional, porcentagem de indivíduos mortos, porcentagem de indivíduos com ovos, e porcentagem de indivíduos hermafroditas. A variação climática foi estimada pela temperatura máxima, umidade relativa mínima e precipitação na estação chuvosa. A presença de recursos foi inferida mediante inspeção visual na área de coleta.

Métodos.

Área de estudo.

O Distrito Federal (DF) tem uma população estimada em três milhões de pessoas e uma densidade populacional média de 444,6 habitantes/km^2. Com uma área total de cinco milhões de quilômetros quadrados e uma área urbana de ~2000km^2, o DF está dividido em 33 regiões administrativas (https://www.df.gov.br/category/sobre-brasilia/). O clima é tropical, com temperatura média de 22°C±10°C e umidade relativa do ar muito variável ao longo do ano. As estações chuvosas e secas são marcadas pela presença de chuva e pela porcentagem de umidade relativa. Geralmente, a estação chuvosa dura de 7 a 8 meses, começando no final de setembro e terminando entre abril e maio, sendo dezembro-janeiro o período mais chuvoso. A estação seca dura 4-5 meses, começando em maio e terminando em setembro, sendo julho-agosto o período menos chuvoso e mais seco (https://www.df.gov.br/333/).

Coleta de indivíduos.

Por propósitos práticos (Jax, 2006), e na ausência de estudos genéticos populacionais ou barreiras evidentes ao fluxo gênico, neste trabalho consideramos os indivíduos de *Lissachatina fulica* dentro da delimitação do Distrito Federal no período de 2021-2022 como uma única população. Considerando que a dispersão do caracol gigante africano pode ser associada ao homem (Adhikari et al., 2020; Vijayan et al., 2020), o sistema rodoviário e ações das pessoas como coletar indivíduos e deixar nos córregos (observação pessoal) certamente contribuem para o fluxo gênico dentro da área do DF. Portanto, assumindo uma população e pela dificuldade em determinar o grau de dispersão passiva de *L. fulica*; a independência entre os pontos de coleta foi garantida com uma distância mínima de 1,5km entre eles, em linha reta.

Foram realizadas 25 campanhas de coleta: 13 em 2021 (4 de fevereiro a 13 de maio) e 12 em 2022 (24 de janeiro a 6 de abril). As coletas foram feitas de forma manual e intensiva. De cada uma participaram de duas a três pessoas, que procuraram ativamente caracóis em uma área de 50m*50m, tendo como centro o local georeferenciado. Cada coletor, usando luvas e carregando um recipiente plástico, percorreu toda a área de coleta procurando indivíduos de *L. fulica* em todos os lugares, vivos ou mortos (conchas vazias, mortos naturalmente, ignorando aqueles que foram coletados pelas pessoas anteriormente). O contato com as pessoas nos pontos visitados foi estabelecido em colaboração com o Instituto Brasília Ambiental (IBRAM) (Figura 1).

Os indivíduos coletados foram colocados em uma caixa plástica de 25L para serem transportados ao laboratório (Figura 1). Em seguida, foram tomadas as coordenadas geográficas. Para definir a presença/ausência de recursos, foram identificados potenciais recursos de refúgio e alimento em todos os pontos. Para identificar os recursos, em cada ponto de coleta foram feitas inspeções visuais em campo. Para complementar a observação em campo, a área de coleta foi fotografada uniformemente.

Figura 1. Processo de coleta de indivíduos de *Lissachatina fulica* no Distrito Federal. Cada foto ilustra: a chegada ao local onde o molusco foi registrado, a busca dos indivíduos com luvas e recipientes plásticos e o recipiente final onde os indivíduos foram depositados para transportar ao laboratório.

Procedimentos no laboratório.

Os indivíduos coletados foram processados no Laboratório de Biologia Evolutiva do Departamento de Genética e Morfologia do Instituto de Ciências Biológicas da Universidade de Brasília. Nesse ambiente, eles foram contados, classificados como vivos ou mortos (conchas vazias ou com individuo sem vida), medidos, pesados e dissecados. O comprimento e largura da concha, e a espessura do peristômio foram medidos com paquímetro digital (±0,01mm) (Figura 2). O peso dos indivíduos vivos foi tomado com uma balança analítica (±0,001g). Como a espécie é hermafrodita protândrica, ou seja, desenvolve primeiro o sistema reprodutivo masculino e depois o feminino (Tomiyama, 1996), buscamos determinar o comprimento da concha quando os indivíduos de *L. fulica* produzem ovos. Para tanto, indivíduos maiores que 30mm foram quebrados, retirados da concha, inspecionados para verificar se tinham ovos e, em caso positivo, esses foram contabilizados (Figura 2). Após esses procedimentos, eles foram sacrificados e colocados em água sanitária a 10% durante uma semana. A cada semana, os indivíduos foram autoclavados e descartados pela universidade como lixo biológico.

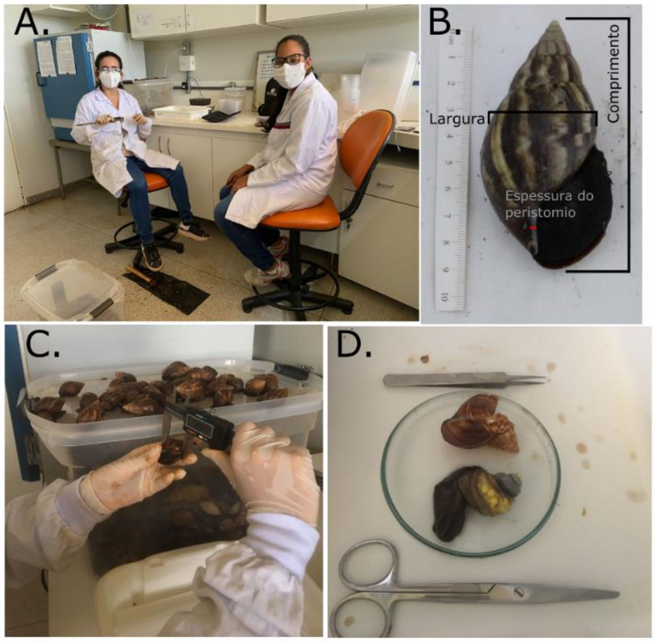

Figura 2. Procedimentos laboratoriais nos indivíduos *Lissachatina fulica* coletados no campo. A. Trabalho conjunto com uma voluntária do Instituto Brasília Ambiental. B. Medições realizadas: comprimento e largura da concha, e espessura do peristômio. C. Medição de uma concha com o paquímetro. D. Caracol grávido, pronto para a contabilização de ovos.

Devido à pandemia da COVID-19, os laboratórios apresentaram limitações de acesso durante os períodos de coleta. Portanto, em sítios amostrais com mais de 100 indivíduos (exceto P16, coletados junto com P17), o comprimento e largura total da concha foram medidos em pelo menos 25% dos caracóis e em todos os intervalos de tamanho. O peso foi medido apenas para indivíduos vivos e aferidos para comprimento e largura. Finalmente, a espessura do peristômio foi medida em indivíduos dissecados e algumas conchas vazias (Tabela 1).

Tabela 1. Número de indivíduos coletados por sítio amostral, e o número de indivíduos com medidas de comprimento e largura, peso, número de ovos por indivíduo e espessura do peristômio. Abundância: Número total de indivíduos coletados, Tamanho: Número de indivíduos com comprimento e largura aferidos, Peso: Número de indivíduos pesados, Ovos: Número de indivíduos que foram dissecados para a busca de ovos.

Data	**Latitude**	**Longitude**	**Sítio Amostral**	**Abundância**	**Tamanho**	**Peso**	**Ovos**	**Espessura peristômio**
04/02/2021	-15,88	-47,77	P1	83	83	19	19	69
12/02/2021	-15,88	-47,75	P2	33	33	24	24	25
17/02/2021	-15,81	-47,78	P3	830	382	360	256	216
24/02/2021	-15,71	-47,86	P4	25	25	16	15	21
02/03/2021	-15,88	-47,99	P5	44	44	39	36	36
03/03/2021	-15,75	-47,84	P6	36	36	31	30	34
12/03/2021	-15,72	-47,86	P7	194	135	115	110	109
17/03/2021	-15,66	-47,84	P8	94	94	88	66	79
19/03/2021	-15,67	-47,85	P9	158	123	103	79	98
30/04/2021	-15,95	-47,83	P10	25	25	13	9	9
03/05/2021	-15,84	-48,02	P11	113	113	108	41	47
07/05/2021	-15,78	-47,93	P12	47	47	26	14	19
13/05/2021	-15,64	-47,80	P13	345	113	108	26	38
24/01/2022	-15,79	-47,84	P14	281	279	174	162	162
28/01/2022	-15,82	-48,02	P15	40	40	35	32	33
01/02/2022	-15,88	-47,79	P16	87	18	18	16	16
01/02/2022	-15,85	-48,02	P17	242	218	140	96	101
07/02/2022	-15,73	-48,16	P18	463	148	76	60	58
09/02/2022	-15,74	-47,76	P19	114	108	102	58	76
14/02/2022	-15,66	-47,80	P20	117	67	67	19	25
16/02/2022	-16,03	-48,05	P21	132	110	80	81	82
21/02/2022	-15,85	-48,12	P22	702	179	147	114	138
21/03/2022	-15,63	-47,66	P23	669	368	322	60	70
28/03/2022	-15,76	-48,11	P24	183	124	102	80	91
06/04/2022	-15,93	-48,23	P25	185	85	69	40	50
			Total	**5242**	**2997**	**2382**	**1543**	**1702**

Análises estatísticas.

Padrões climáticos espaciais e temporais. Para as análises climáticas foram selecionadas a temperatura máxima, umidade relativa mínima e precipitação. Estas variáveis foram selecionadas com base no trabalho de Albuquerque et al. (2009), no qual, para uma população em Lauro de Freitas (BA), a faixa de temperatura, umidade e precipitação influenciaram comprimento, peso e fator de condição de *L. fulica*. Os dados para cada data de coleta foram obtidos da estação meteorológica de Brasília do Instituto Nacional de Meteorologia (INMET) (https://tempo.inmet.gov.br/CondicoesRegistradas). A seleção da estação de Brasília foi baseada na comparação gráfica das variáveis climáticas das estações meteorológicas de Brasília, Brazlândia e Gama do DF para um mesmo período (Figura 3).

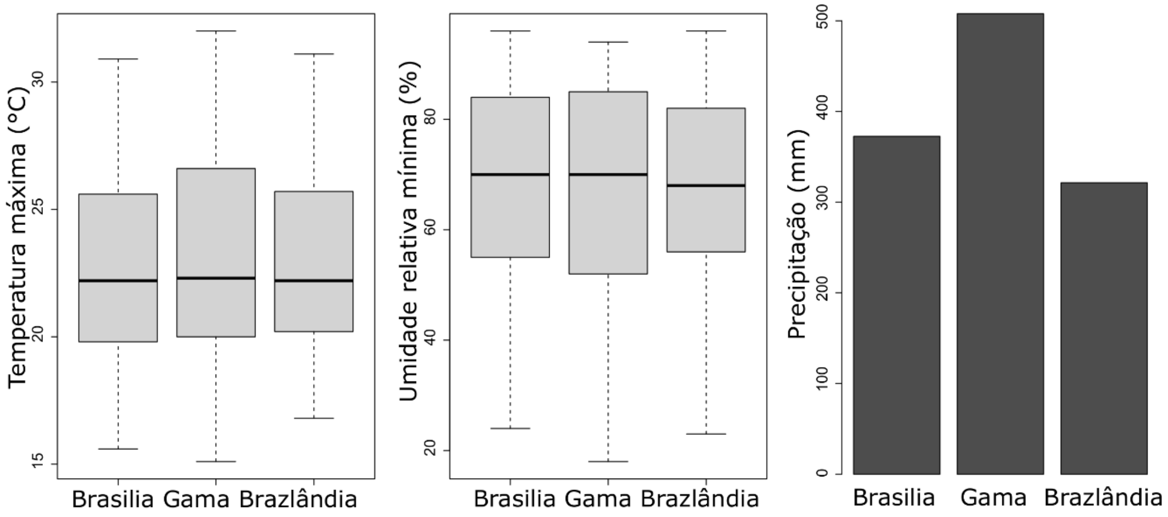

Figura 3. Variação da temperatura máxima (°C), umidade relativa mínima (%) e precipitação (mm) nas estações meteorológicas de Brasília, Brazlândia e Gama do DF, no período de 15 de janeiro a 15 de abril de 2022.

Caracterização da população. Para caracterizar a população de *Lissachatina fulica*, foram usados a abundância, comprimento e largura da concha, peso, espessura do peristômio e número de ovos por indivíduo. Para abundância, foi feito um mapa com o número de indivíduos coletados em cada ponto, usando os pacotes rgdal (Bivand et al., 2022) e ggplot2 (Wickham, 2016) do programa R 4.2.0. A fim de entender a distribuição de valores do comprimento, largura e peso se estimaram média, desvio padrão e mediana dos indivíduos medidos. Para estabelecer o grau de alometria na população de *L. fulica* no DF, a largura e o peso foram associados ao comprimento, usando um modelo linear e potencial. Todas estas análises foram realizadas com o software R 4.2.0.

Para investigar o potencial reprodutivo da população, foram investigadas seguintes características relacionadas aos ovos: porcentagem de indivíduos grávidos com o intervalo de comprimento da concha onde se encontraram, número total de ovos, e número de ovos por indivíduo (mínimo, máximo, média, desvio padrão e mediana). Como o comprimento da concha e a espessura do peristômio têm sido usados como indicadores de período reprodutivo em *L. fulica* (Tomiyama & Miyashita, 1992; Tomiyama, 1993), essas variáveis foram associadas ao número de ovos mediante analise de correlação de Pearson. Todas estas análises foram realizadas com o software R 4.2.0.

Associações entre variáveis populacionais. Por ponto, foram calculadas a densidade populacional, porcentagem de indivíduos mortos, porcentagem de indivíduos com ovos e porcentagem de indivíduos hermafroditas. A densidade populacional foi estimada como número de indivíduos/m^2. A porcentagem de indivíduos mortos foi estimada como número de indivíduos mortos/total de indivíduos, multiplicado por 100. A porcentagem de indivíduos com ovos foi estimada como número de indivíduos grávidos/total de indivíduos, multiplicado por 100. A porcentagem de indivíduos hermafroditas foi estimada como número de indivíduos com comprimento maior do que o menor indivíduo grávido/total de indivíduos, multiplicado por 100. A fim de entender a distribuição de valores das variáveis foram calculados os valores mínimos, máximos, média, desvio padrão e mediana.

A densidade populacional, porcentagem de indivíduos mortos, porcentagem de indivíduos com ovos e porcentagem de indivíduos hermafroditas por ponto foram associadas mediante correlação de Pearson. Variáveis com coeficiente de correlação significativos foram empregadas em modelos lineares para investigar causalidade, juntamente com a verificação gráfica de normalidade e homogeneidade da variância. Todas estas análises foram realizadas com o programa R 4.2.0 (Material Suplementar 1 e 2).

Associações com variáveis climáticas. A temperatura máxima, umidade relativa mínima e precipitação foram calculadas para cada coleta, com base nos valores da estação meteorológica de Brasília de 04 de janeiro a 13 de maio de 2021 e 24 de dezembro de 2021 a 6 de abril de 2022. Como os valores de temperatura e umidade não seguem uma distribuição normal (teste kolmogorov smirnoff $p< 0,001$), a mediana foi calculada para os 8, 15 e 30 dias antes de cada coleta. No caso da precipitação, se somaram os valores de precipitação para os 8, 15 e 30 dias antes de cada coleta. A estação metereológica de Brasilía foi usada já que não foram observadas diferenças entre os valores climáticos com as outras estações (Gama, Brazlândia).

A densidade populacional, porcentagem de indivíduos mortos, porcentagem de indivíduos com ovos e porcentagem de indivíduos hermafroditas foram associadas com as variáveis climáticas de 8, 15 e 30 dias mediante correlação de Pearson. Variáveis com coeficiente de correlação significativos foram empregadas em modelos lineares para investigar causalidade, juntamente com a verificação gráfica de normalidade e homogeneidade da variância. Todas estas análises foram realizadas com o programa R 4.2.0 (Material Suplementar 1 e 2).

Associações com variáveis de recursos. Com base nas observações em campo e nas fotografias, os recursos foram qualitativamente definidos como: grama (baixa ou alta), concreto (estradas ou pavimentos), paredes (ocas, tijolo limpo ou pintadas), lixo orgânico e inorgânico, detritos, plantas (ornamentais ou agrícolas), solo, areia, entulho e serapilheira. As fotografias de cada ponto e as observações em campo foram usadas a fim de registrar a presença/ausência de cada recurso nos pontos. No caso de área construída, o cálculo foi da porcentagem de área construída por ponto. Para tanto, foi estabelecido um quadrado de 200m*200m no programa Google Earth pro, sendo o centro a coordenada geográfica tomada no campo (ver coleta de indivíduos). Dentro do quadrado, foram criados polígonos menores cobrindo a área construída. Após o cálculo da área de cada polígono, a porcentagem de área construída foi calculada somando as áreas de cada polígono e dividindo sobre a área total, multiplicando por 100. Os valores: mínimo, máximo, média, desvio padrão e mediana foram calculados para entender o intervalo da porcentagem de área construída onde se encontrou *L. fulica*.

Para determinar a influência da presença ou ausência de um determinado recurso na densidade populacional, porcentagem de indivíduos mortos, porcentagem de indivíduos com ovos e porcentagem de indivíduos hermafroditas, foram executados modelos lineares, juntamente com a verificação gráfica de normalidade e homogeneidade de variância. Os pontos com área construída superior a 50% foram categorizados como "Alta" e pontos com área construída inferior a 50% foram categorizados como "Baixa". Estas análises foram realizadas com o programa R 4.2.0 (Material Suplementar 1 e 2).

Resultados.

Caracterização da população

Um total de 5242 indivíduos de *L. fulica* foi coletado (Tabela 1), 2027 em 2021 e 3215 em 2022. A abundância nos pontos de coleta variou de 25 (pontos 4 e 10) a 830 indivíduos (ponto 3).

A maioria dos pontos tiveram abundâncias menores que 500 indivíduos (22 de 25 pontos), e apenas 3 pontos tiveram mais de 500 indivíduos. O caracol gigante africano foi encontrado ao longo da zona urbana do DF e os pontos com mais de 500 indivíduos não estão próximos no espaço. Portanto, o caracol gigante africano está amplamente distribuído na área urbana do DF (Figura 4).

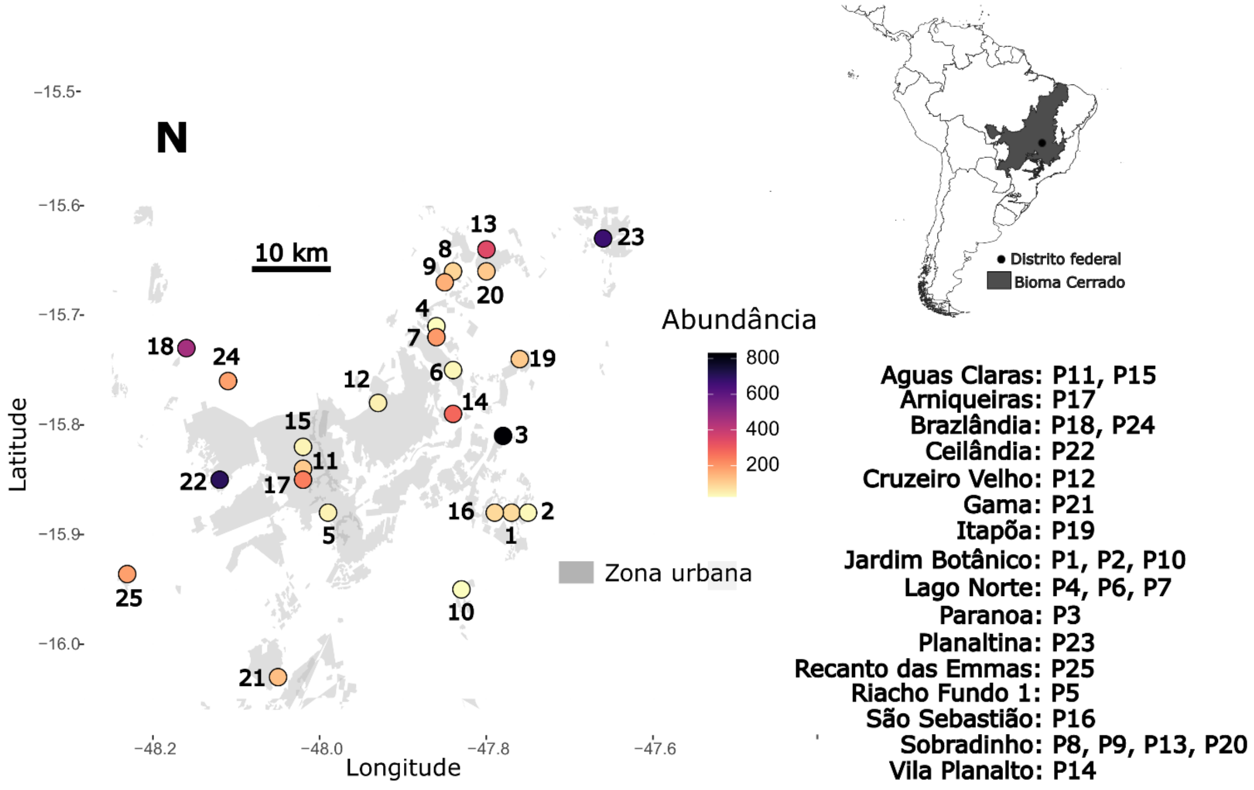

Figura 4. Distribuição espacial da abundância do caracol gigante africano (*Lissachatina fulica*) nos 25 pontos de coleta do DF. A direita o mapa com a localização do DF no bioma Cerrado e as Regiões Administrativas onde estão localizados os pontos.

Os indivíduos coletados apresentaram variação de tamanho e peso. O comprimento da concha variou de 4,17mm a 163mm (média 50,01±20,88mm, mediana 55,26mm) e a largura de 3,07mm a 60mm (média 24,85±9,03mm, mediana 27,47mm). O peso dos espécimes variou de 0,043g a 131,657g (média 9,46±16,22g, mediana 19,05g). No comprimento da concha foi observada uma tendência de distribuição bimodal que reflete em parte os indivíduos que não colocam (0-50mm) e os que colocam ovos (50-100mm) (Figura 5A). O peso tem uma distribuição assimétrica à esquerda

(Figura 5B). A variação no tamanho dos indivíduos *L. fulica* no DF indica que pelo menos metade dos indivíduos tem potencial de postura de ovos.

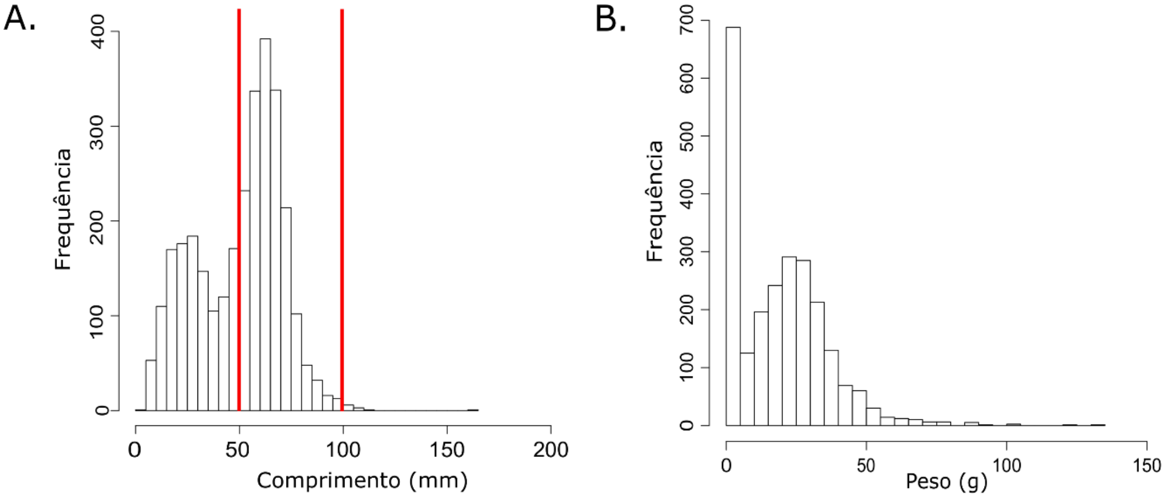

Figura 5. Distribuição de frequência do comprimento da concha (A) e do peso (B) dos indivíduos de *Lissachatina fulica* coletados no DF A. Distribuição de frequência do comprimento total da concha (mm) (n=2997), o intervalo entre as linhas vermelhas indica a gama de indivíduos com ovos. B. Distribuição de frequência de peso (g) em indivíduos (n=2382).

Apenas um entre os indivíduos coletados apresentou concha levogira, em contraste com o resto, que apresentou concha dextrogira. O padrão levogiro foi identificado pela posição da abertura oposta à dos outros indivíduos (Figura 6A e 6B). Com uma frequência de 1 em 5242, pode ser estimada uma probabilidade de 0,0002% de ocorrência desta condição na população (Figura 6). O indivíduo foi encontrado vivo com um comprimento da concha de 68,08mm e largura da concha de 33,48mm, pelo qual é possível que possa colocar ovos. As outras medidas tomadas foram: comprimento da abertura: 36,35mm, largura da abertura: 20,38mm e peso: 35,137g.

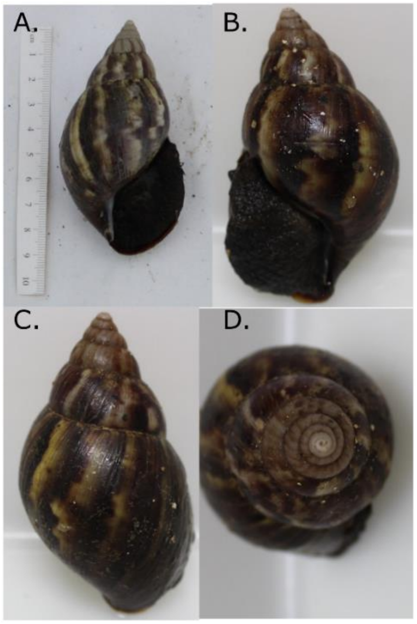

Figura 6. Identificação de indivíduo de *Lissachatina fulica* levogiro coletado no ponto 17 em fevereiro de 2022. A. Vista ventral de um indivíduo dextrogiro. B. Vista ventral do indivíduo levogiro mostrando a abertura da concha no lado oposto ao indivíduo dextrogiro. C. Vista dorsal do indivíduo levogiro. D. Vista apical do indivíduo levogiro.

As associações entre a largura e o peso com o comprimento da concha refletem alometria. A largura aumenta 0,43 vezes com o comprimento (Figura 7A). Entre o peso e o comprimento existe uma tendência potencial, onde o peso aumenta em diferentes velocidades dependendo do intervalo de tamanho. O coeficiente potencial "b" da associação entre o peso e o comprimento, que neste caso foi de 2,64, indica uma relação alométrica negativa (Figura 7B).

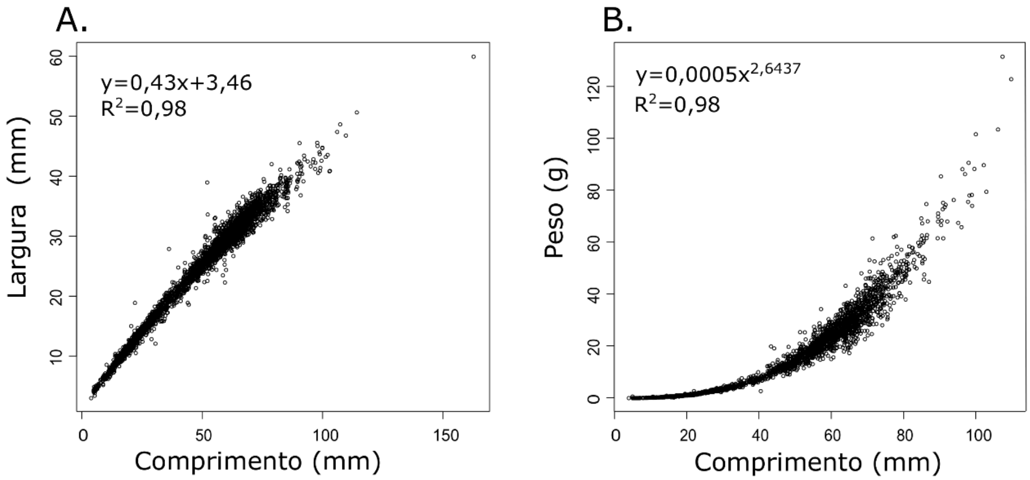

Figura 7. Associações entre o tamanho da concha e o peso dos indivíduos de *Lissachatina fulica* coletados no Distrito Federal. A. Largura (mm) em função do comprimento (mm) (n=2972). B. Peso (g) em função do comprimento (mm) (n=2387).

Com relação aos aspectos reprodutivos, 11,2% dos indivíduos examinados estavam grávidos; o comprimento de suas conchas variou de 50,05mm até 103,14mm. Com um total de 14893 ovos, o número de ovos por indivíduo variou de 3 até 246 ovos (média 86,58±40,80 ovos/indivíduo, mediana 82 ovos/indivíduo) (Figura 8A). O número de ovos por indivíduo apresenta uma correlação positiva moderada com o comprimento da concha ($r=0,44$, $t_{(170)}=6,43$, $p<0,01$), se observando mais variação a partir de 80mm (Figura 8B). Por outro lado, a espessura do peristômio variou de 0,8mm a 7mm (média 2,54±0,66mm, mediana 2,49mm), e não apresentou correlação com o número de ovos (Figura 8C).

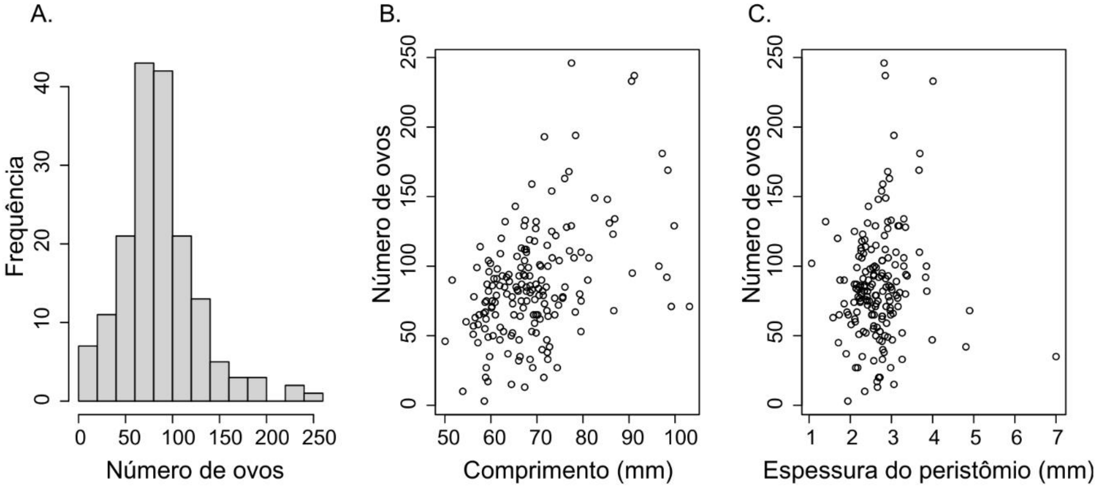

Figura 8. Atributos reprodutivos do caracol gigante africano (*Lissachatina fulica*) no DF. A. Frequência de número de ovos por indivíduo (n=172). B. Número de ovos por indivíduo associados ao comprimento da concha do caracol (n=172). C. Número de ovos por indivíduo associados à espessura do peristômio (n=169).

Associações entre variáveis populacionais.

Em cada ponto foi calculada a densidade populacional (ind/m^2) e as porcentagens de indivíduos mortos, indivíduos com ovos e hermafroditas. A densidade populacional variou de 0,01ind/m^2 a 0,332ind/m^2 (média 0,084±0,09ind/m^2, mediana 0,046ind/m^2). A porcentagem de indivíduos mortos variou de 1,45% a 74,7% (média 28,7±19,4%, mediana 32,4%). A porcentagem de indivíduos com ovos variou de 0% a 30% (média 6,2±8,3%, mediana 2,6%). A porcentagem de indivíduos hermafroditas variou de 12,9% a 89,4% (média 61,2±24,34%, mediana 67,1%). A porcentagem de indivíduos com ovos apresentou uma correlação positiva moderada com a porcentagem de indivíduos hermafroditas e uma correlação negativa fraca com a densidade populacional (marginalmente significante); as demais correlações não foram significativas (Tabela 2).

Tabela 2. Coeficientes de correlação de Pearson entre variáveis de *L. fulica*. (·)valor p < 0,1, (*) valor p < 0,01. (n = 25)

	Densidade (Ind/m^2)	**Indivíduos mortos (%)**	**Indivíduos com ovos (%)**
Indivíduos mortos (%)	0,03		
Indivíduos com ovos (%)	-0,33·	-0,22	
Indivíduos hermafroditas (%)	-0,08	0,14	0,62*

O modelo linear aponta que a porcentagem de indivíduos com ovos é influenciada pela densidade populacional, pela porcentagem de indivíduos mortos, e pela porcentagem de hermafroditas (R^2=0,50, $F_{(3,21)}$=9,18, p=0,0004). A porcentagem de indivíduos com ovos diminui com o incremento da densidade populacional (estimador=-24,7±13,2, p=0,07) (Figura 9A) e da porcentagem de indivíduos mortos (estimador=-0,13±0,06, p=0,04) (Figura 9B). Ao contrário, a porcentagem de indivíduos com ovos está positivamente relacionada com a porcentagem de indivíduos hermafroditas (estimador=0,2±0,04, p=0,0002) (Figura 9C) (Material suplementar 2). Portanto, o recrutamento da população seria alto com menor densidade populacional, menor mortalidade e mais indivíduos hermafroditas.

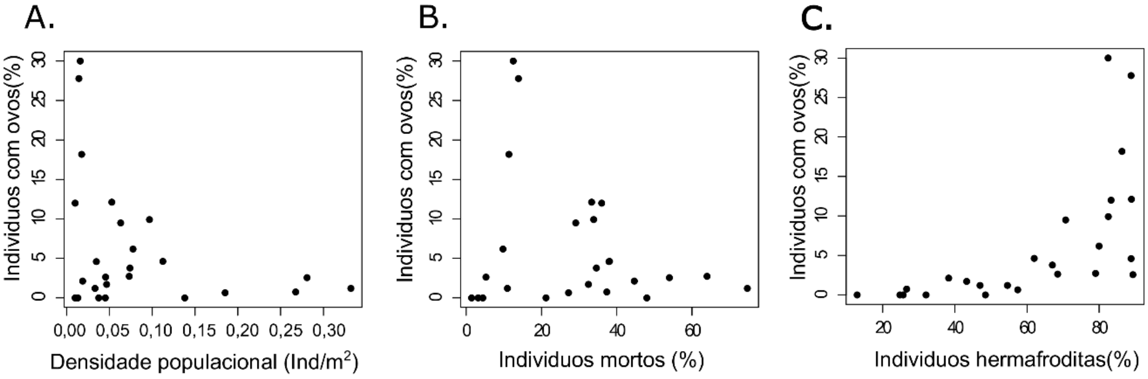

Figura 9. Associações entre a porcentagem de indivíduos com ovos com (A) densidade populacional, (B) porcentagem de indivíduos mortos e (C) porcentagem de indivíduos hermafroditas de *Lissachatina fulica* (n=25).

Associações com variáveis climáticas.

A mediana de temperatura máxima variou de 19,7°C a 24,15°C nos 8 dias que antecederam as coletas, de 20°C a 23,45°C em 15 dias, e de 20,4°C a 22,8°C em 30 dias. A mediana de umidade relativa mínima variou de 54% a 89,5% (8 dias), de 57,5% a 88,5% (15 dias), e de 64% a 84% (30 dias). A precipitação mostrou máximas de 180mm/8 dias, 364,8mm/15 dias e 616mm/30 dias. Nos três cenários analisados (8, 15 e 30 dias), a porcentagem de indivíduos mortos teve uma correlação positiva moderada com a temperatura máxima, e uma correlação negativa fraca (marginalmente significante) com a umidade relativa mínima. No período de 30 dias, a porcentagem de indivíduos com ovos teve uma correlação positiva moderada com a umidade relativa mínima, e uma correlação positiva fraca (marginalmente significante) com a precipitação (Tabela 3).

Tabela 3. Coeficientes de correlação de Pearson entre a densidade populacional, porcentagens de indivíduos mortos, com ovos e hermafroditas e as variáveis climáticas de 8, 15 e 30 dias. Mediana da temperatura máxima, porcentagem mínima de umidade relativa e a soma da precipitação para os 8, 15 e 30 dias antes de cada coleta. ($^{\cdot}$)valor de p < 0,1, (*) valor de p < 0,05, (**) valor de p < 0,01

		Densidade (Ind/m^2)	**Indivíduos mortos (%)**	**Indivíduos com ovos (%)**	**Indivíduos hermafroditas (%)**
8 dias	Temperatura máxima	0,05	0,42*	-0,01	0,01
	Umidade relativa mínima	0,06	-0,34$^{\cdot}$	0,1	0,17
	Precipitação	0,3	-0,21	-0,12	0,04
15 dias	Temperatura máxima	-0,02	0,42*	0,07	0,17
	Umidade relativa mínima	0,13	-0,35$^{\cdot}$	0,14	0,21
	Precipitação	0,15	-0,12	0,03	0,09
30 dias	Temperatura máxima	0,15	0,52**	-0,28	0,03
	Umidade relativa mínima	-0,11	-0,38$^{\cdot}$	0,52**	0,39
	Precipitação	-0,02	-0,26	0,35$^{\cdot}$	0,25

Os modelos lineares mostram que, considerando o clima nos 30 dias que antecederam cada coleta, a porcentagem de indivíduos mortos foi influenciada pela temperatura máxima e umidade relativa mínima (R^2=0,21, F$_{(2,22)}$=4,19, p=0,03). Marginalmente significante, a porcentagem de indivíduos mortos aumenta junto com a temperatura máxima (estimador=12,6±6,4, p=0,06) (Figura 10A). Ao contrário, embora não significativo, a porcentagem de indivíduos mortos diminui com o aumento da umidade relativa mínima (estimador=-0,3±0,7, p=0,71) (Figura 10B). Por sua vez, a porcentagem de indivíduos com ovos é influenciada pela umidade relativa mínima e a precipitação (R^2=0,21, F$_{(2,22)}$=4,28, p=0,02). A porcentagem de indivíduos com ovos aumenta junto com a umidade relativa mínima (estimador=0,6±0,3, p=0,04) (Figura 10C) e, embora não significativo, também com a precipitação (estimador=0,003±0,01, p=0,74) (Figura 10D) (Material Suplementar 2). Portanto, a variação de temperatura, umidade e precipitação na estação chuvosa influenciam a população de *L. fulica*.

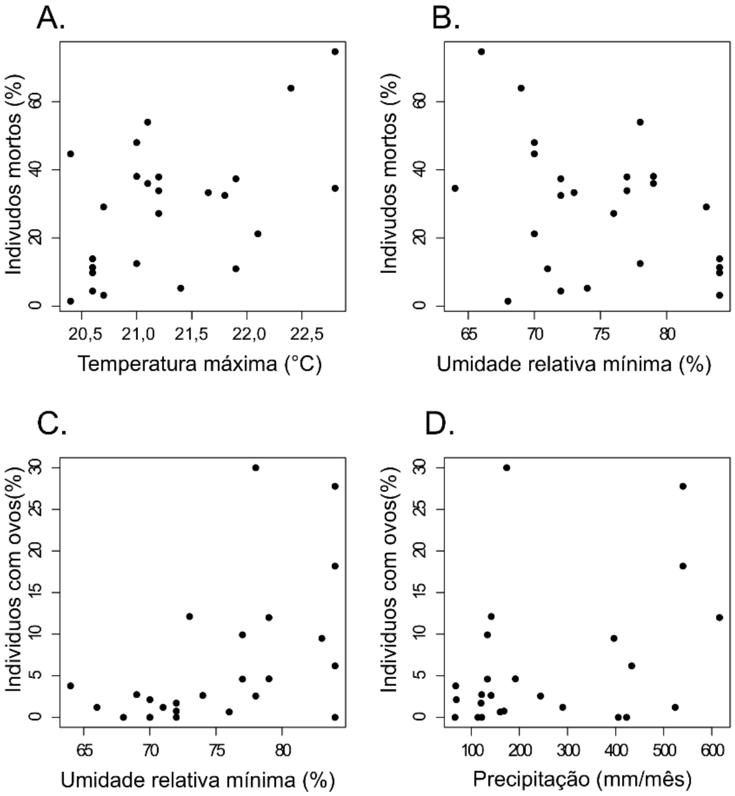

Figura 10. Associações entre as porcentagens de indivíduos mortos e indivíduos com ovos com variáveis climáticas de 30 dias (n=25). A. Porcentagem de indivíduos mortos vs. temperatura máxima. B. Porcentagem de indivíduos mortos vs. umidade relativa mínima. C. Porcentagem de indivíduos com ovos vs. umidade relativa mínima. D. Porcentagem de indivíduos com ovos vs. Precipitação.

Associações com variáveis de recursos.

Os indivíduos de *L. fulica* se encontraram principalmente em propriedades privadas, e às vezes em parques públicos e áreas verdes. Nos 25 pontos, foram encontrados elementos como grama (baixa ou alta), concreto (estradas ou pavimentos), paredes (ocas, tijolo limpo ou pintadas), lixo orgânico e inorgânico, plantas (ornamentais ou cultivadas), solo, areia, serapilheira ou entulho. Todos os pontos tinham grama, 17 pontos tinham concreto, 12 pontos tinham paredes e solo, 11 pontos tinham plantas e todos os outros recursos estiveram em menos de 10 pontos. O ponto com a maior variedade de recursos foi o ponto 22 com 8 recursos, seguido pelo ponto 25 com 7 recursos e o ponto 3 com 6 recursos. Durante as coletas, os indivíduos fizeram uso de paredes, plantas e solo (Figura 11).

Figura 11. Locais onde foram encontrados indivíduos de *Lissachatina fulica* no Distrito Federal, mostrando alguns dos recursos que podem ser utilizados, tais como bananeiras (*Musa* sp.), paredes, gramíneas altas e hortaliças.

Quando se associam a densidade populacional, as porcentagens de indivíduos mortos, com ovos, e hermafroditas, com a presença e ausência de recurso, apenas a densidade populacional tem uma relação significativa com o solo (R^2=0,15, $F_{(1,23)}$= 5,304, p=0,03) (Figura 12A). Embora marginalmente significativo, o lixo também pode estar influenciando a densidade populacional (R^2=0,10, $F_{(1,23)}$= 3,807, p=0,06) (Figura 12B). Da mesma forma, a porcentagem de indivíduos hermafroditas pode estar sendo influenciada pelo concreto (R^2=0,09, $F_{(1,23)}$= 3,607, p=0,07) e pelo solo (R^2=0,08, $F_{(1,23)}$= 3,109, p=0,09) (Tabela 4). Portanto, a densidade populacional e a porcentagem de indivíduos hermafroditas podem estar sendo influenciadas pela presença/ausência de solo, lixo e concreto.

Tabela 4. Valores de F com 1, 23 graus de liberdade dos modelos lineares entre a densidade populacional, porcentagens de indivíduos mortos, com ovos e hermafroditas com a presença e ausência dos recursos de concreto, parede, lixo, entulho, plantas, solo e área construída. (*) Valor de $p<0,05$

	Concreto	**Parede**	**Lixo**	**Entulho**	**Plantas**	**Solo**	**Area construída**
Densidade (Ind/m^2)	0,01	1,72	3,81	0,38	0,87	5,30*	0,0004
Indivíduos mortos (%)	0,08	1,12	0,14	0,26	0,05	0,16	0,76
Indivíduos com ovos (%)	1,41	0,66	1,26	0,07	0,15	0,01	0,51
Indivíduos hermafroditas (%)	3,61	0,03	0,13	1,71	0,03	3,11	4,37*

A área construída ao redor dos pontos de coleta variou de 11,27% a 90,92% (média 48,88±27,51%, mediana 52,45%) e influenciou a porcentagem de indivíduos hermafroditas (R^2= 0,12, $F_{(1,23)}$= 4,37, p=0,047) (Tabela 4). Lugares com baixa construção (<50%) apresentam porcentagens altas de indivíduos hermafroditas com menor variação, em contraste com lugares com alta construção (>50%) (Figura 12C). A porcentagem da área construída não influencia a densidade populacional, a porcentagem de indivíduos mortos e a porcentagem de indivíduos com ovos (Tabela 4). Portanto, o caracol gigante africano pode ser encontrado em locais com ampla gama de áreas construídas, mas com diferentes frequências de tamanho.

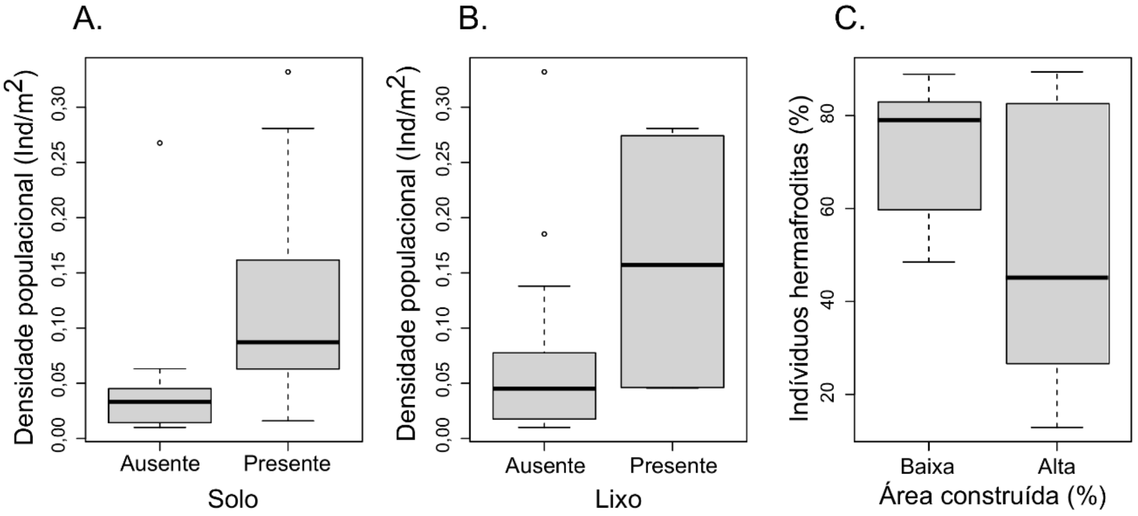

Figura 12. Associações entre a densidade populacional com a presença/ausência de solo (A) e lixo (B), e a porcentagem de indivíduos hermafroditas com a área construída (C) nos pontos de coleta do DF (n=25).

Discussão.

Caracterização da população

Este trabalho apresenta a primeira caracterização ecológica de *Lissachatina fulica* no Distrito Federal, uma unidade federativa do Brasil onde o molusco está presente há mais de uma década (Cazarin-Oliveira et al., 2021). Espacialmente, a população de *L. fulica* no DF está presente na área urbana e amplamente distribuída, indicando uma população bem estabelecida. Por outro lado, o individuo levogiro representa um achado interessante. Embora os gastrópodes exibam padrões de crescimento dextrogiro e levogiro, o padrão levogiro é menos comum (Lesoway & Henry, 2021). Apenas quatro localidades registraram a condição levogira em *L. fulica*: Belém (PA), Madagascar, Austrália e Filipinas (Santos et al., 2019). Como população estabelecida, a caracterização ecológica é importante para o manejo, identificação e mitigação do impacto.

Os indivíduos de *L. fulica* no DF mostram variação no comprimento, largura e peso. A maior concha teve um comprimento de 163mm e os indivíduos vivos atingiram até 100mm de comprimento. O maior comprimento registrado na literatura é de 200mm (Barçante, 2005). Entretanto, a maioria dos valores de comprimento não excedem os 150mm (Vasconcellos & Pile, 2001). A largura da concha de *L. fulica* apresenta um menor crescimento em relação às populações da Colômbia ($y=0,46x+4,25$; (Avendaño & Linares, 2015)), Equador ($y=0,48x+4,26$; (Villavicencio-Abril et al., 2020)) e Amazônia Brasileira ($y=0,486x+3,204$; (Santos et al., 2020)). O peso de *L. fulica* segue uma relação potencial com o comprimento, como também foi observado em Lauro de Freitas (BA) ($y=0,0006x^{2,60}$; Albuquerque et al., 2009). Assim, pode-se dizer que os indivíduos *L. fulica* no DF apresentam tamanhos menores e uma relação alométrica negativa com o peso.

As classes etárias de *L. fulica* são definidas pelo comprimento da concha (Simião & Fischer, 2004) ou pela espessura do peristômio (Tomiyama, 1993). Devido à falta de conhecimento da ecologia reprodutiva de *L. fulica* se verificou qual destas medidas é mais eficaz estimando a classe hermafrodita. O número de ovos esteve correlacionado com o comprimento, mas não com a espessura do peristômio. As medições do peristômio foram baseadas no esquema de Silva & Omena (2014), mas muitos dos indivíduos excederam as medições da literatura (Tomiyama, 1993; Silva & Omena, 2014; Dickens et al., 2018a). Assim, o comprimento total da concha é um bom estimador da classe hermafrodita desde que seja apoiado por dados reprodutivos; ou até que seja encontrada uma maneira mais eficaz de designar a classe de idade em *L. fulica*.

O intervalo de comprimento dos indivíduos com ovos pode indicar o potencial reprodutivo. Neste trabalho, esse intervalo foi de 50,05mm-103,4mm, o que parece pouco em comparação com a população dos EUA (48mm-128mm) (Roda et al., 2016). A diferença entre os intervalos pode ser devida ao menor tamanho dos indivíduos no DF pela sazonalidade, ou a diferenças no desenho experimental. Partindo do princípio de que *L. fulica* põe ovos desde os 4 meses (124 dias) (Dickens et al., 2018a), os indivíduos nascidos em outubro (início da chuva) começariam a pôr ovos a partir de janeiro. Assim, *L. fulica* teria apenas 3-4 meses para investir no crescimento e na reprodução até a próxima estação seca. Como a maturidade sexual pode diferir (exemplo, Colômbia 31,42 mm (De la Ossa et al., 2017), Cuba 70mm (Vázquez et al., 2018)) saber quando os indivíduos são reprodutivos no campo é importante para o manejo.

O potencial reprodutivo de *L. fulica* no DF também foi medido pelo número máximo de ovos por indivíduo, o número médio de ovos por indivíduo e a porcentagem total de indivíduos com ovos. O número máximo de ovos por indivíduo foi 246 ovos, quase metade do recorde de 460 ovos (Roda et al., 2016). O número médio de ovos por indivíduo foi 86, bem abaixo dos 113 ovos/indivíduo registrados nos EUA (Roda et al., 2016), mas semelhante aos 80 ovos/ind. observados em Salvador (BA) (Silva & Omena, 2014). Embora possa parecer um número baixo de ovos; a porcentagem de indivíduos com ovos no DF (11,2%) junto com a de pontos com indivíduos grávidos (80%) mantém o recrutamento de *L. fulica* no DF.

Associações entre variáveis populacionais.

A porcentagem de indivíduos de *L. fulica* com ovos está relacionada com a porcentagem de indivíduos hermafroditas, de indivíduos mortos, e talvez também com a densidade populacional. Quando o tamanho dos indivíduos de uma população aumenta, é mais provável observar indivíduos grávidos (Roda et al., 2016), pois os hermafroditas têm mais oportunidades para se acasalar com outros hermafroditas ou machos (Tomiyama, 1996; Tomiyama, 2002). O efeito da porcentagem de indivíduos mortos é provavelmente um reflexo do controle incipiente das pessoas. O método adotado é o controle físico (Thiengo et al., 2007), sendo os indivíduos maiores o alvo mais fácil. Altos valores de densidade populacional diminuem o crescimento e a produção de ovos em populações de laboratório (Dickens et al., 2018a; 2018b). Talvez a densidade populacional registrada no DF seja muito baixa (em comparação com registros >100 ind/m^2 (Gutierrez-Gregoric et al., 2011)) para impactar significativamente o número de indivíduos grávidos. Portanto, para controlar *L. fulica* é importante conhecer o comprimento quando os indivíduos começam a postura de ovos.

Associações com variáveis climáticas.

A temperatura é postulada como uma das variáveis mais influentes no crescimento de *L. fulica* no laboratório (Ahmed & Raut, 1991), bem como na sua abundância em campo (Silva et al., 2022). Na população aqui estudada, a porcentagem de indivíduos mortos respondeu à temperatura máxima em 30 dias. Apesar da temperatura durante as coletas estar dentro da faixa de tolerância (8°C-38°C) e reprodução de *L. fulica* em laboratório (22°C-32°C) (Sharma & Dickens, 2018), temperaturas mais altas parecem afetar a mortalidade do caracol. Ou seja, pode ser que o caracol seja mais sensível à temperatura máxima do que a outros indicadores de temperatura. Também há a possibilidade de que o aumento de indivíduos mortos pela temperatura possa ser devido a aspectos comportamentais. A temperatura influencia os hábitos de repouso ou se enterrar de *L. fulica* (Durço et al., 2013; Silva et al., 2022), o que pode diminuir a quantidade de indivíduos detectáveis em campo. Portanto, a temperatura máxima, como outros fatores, modula a variabilidade da mortalidade de *L. fulica* no campo.

A sazonalidade na precipitação é reconhecida como o principal fator que influencia a atividade reprodutiva em *L. fulica* (Raut & Barker, 2002). A porcentagem de indivíduos com ovos responde com a umidade relativa mínima em 30 dias. A influência da umidade relativa pode ser indireta, pois quanto maior for a precipitação, mais ativa será *L. fulica* sexualmente (Silva & Omena, 2014). E, com maior atividade sexual é possível que o número de indivíduos grávidos aumente. A resposta climática de 30 dias é explicada pelo tempo entre posturas de 21 a 76 dias no período ativo de *L. fulica* (Raut, 1991). Portanto, a variação na porcentagem de indivíduos que colocam ovos na estação chuvosa reflete o tempo do período reprodutivo que prepara a população para a estação seca.

Associações com variáveis de recursos

A densidade populacional responde com a presença de alguns recursos. Os pontos com mais recursos presentes tiveram valores altos de densidade populacional, o que é parcialmente consistente com a literatura que associa o molusco ao ambiente urbano (Albuquerque et al., 2008; Fischer et al., 2008; Almeida et al., 2016; Goldyn et al., 2017). Os tipos de recursos disponíveis que influenciaram a densidade populacional foram o solo e o lixo. O solo teve uma influência significativa na densidade populacional por ser o local de descanso mais frequente (Almeida et al., 2016). Embora a influência do lixo na densidade populacional tenha sido marginalmente significativa, a abundância de *L. fulica* tem sido associada a altas quantidades de resíduos orgânicos e inorgânicos (Fischer et al., 2008; Goldyn et al., 2017). Apesar da baixa densidade populacional de *L. fulica* no DF, este parâmetro continua respondendo aos recursos e, especialmente, à presença de resíduos e locais de repouso.

A porcentagem de indivíduos hermafroditas responde à porcentagem de área construída. A alta porcentagem relativamente constante de hermafroditas em áreas com baixa construção indica que estão atuando como fontes. As áreas com baixas porcentagens de construção são geralmente pequenas plantações (Thiengo et al., 2007; Goldyn et al., 2016) que podem fornecer um recurso constante de alimento e refúgio. Em áreas com altas porcentagens de construção há uma alta variação na porcentagem de indivíduos hermafroditas, pois são mais detectáveis para controle manual. Além disso, a dispersão mediada pelo homem é maior nas cidades (Cadotte et al., 2017; Borden & Flory, 2021), o que pode introduzir indivíduos menores (talvez ovos) nos pontos. Portanto, *L. fulica* responde ao gradiente de construção dentro das áreas urbanas e o controle deve focar nas áreas que podem funcionar como fontes.

Conclusões

O caracol gigante africano está estabelecido no Distrito Federal. Este molusco se torna um problema a cada estação chuvosa, quando se torna perceptível para as pessoas. Com a caracterização ecológica no presente trabalho, o plano de manejo de *L. fulica* ganha subsídios para direcionar ações de coleta. Essas deveriam ocorrer em todo o DF, pois o caracol gigante africano é encontrado desde regiões administrativas altamente construídas até aquelas que tendem a ser rurais. Além disso, a coleta deve ser sistematizada e com maior intensidade no início das chuvas, já que os dados indicam que *L. fulica* é fértil mais precocemente do que o esperado. Considerando o exposto, e a relativamente baixa densidade do caracol no DF, consideramos possível seu manejo nessa unidade da Federação.

No Distrito Federal, a probabilidade de que os indivíduos com comprimento maior do que 50mm coloquem ovos é alta. Assim, o comprimento da concha continua sendo o melhor estimador da faixa etária hermafrodita e da quantidade de ovos. Com este conhecimento reprodutivo é possível saber qual é o momento adequado para realizar coleta do molusco, qual parcela da população está aportando no mantenimento, cada quanto acontece a postura de ovos, como estaria impactando a coleta manual na dinâmica populacional, etc. Portanto, para construir um plano de manejo de uma população de *L. fulica* é necessária a caracterização de seus atributos reprodutivos.

A porcentagem de indivíduos com ovos está associada positivamente com a porcentagem de indivíduos hermafroditas, e negativamente com a porcentagem de indivíduos mortos e densidade populacional (Figura 13). A porcentagem de indivíduos mortos e de indivíduos com ovos respondem a variações no clima (Figura 13). A densidade populacional e a porcentagem de indivíduos hermafroditas respondem à presença de recursos (Figura 13). Portanto, os aspectos de *L. fulica* respondem diferente às variações de clima e presença de recursos. Pode-se dizer que embora a *L. fulica* tenha uma ampla faixa de tolerância climática, seus atributos respondem de forma diferente às variações ambientais. Além disso, *L. fulica* é uma espécie invasora que se beneficia de ambientes urbanos, mas este benefício depende do atributo. Assim, as respostas de *L. fulica* devem avaliar-se em sinergia, entendendo como suas populações são mantidas, para quantificar seus impactos e aperfeiçoar seu manejo.

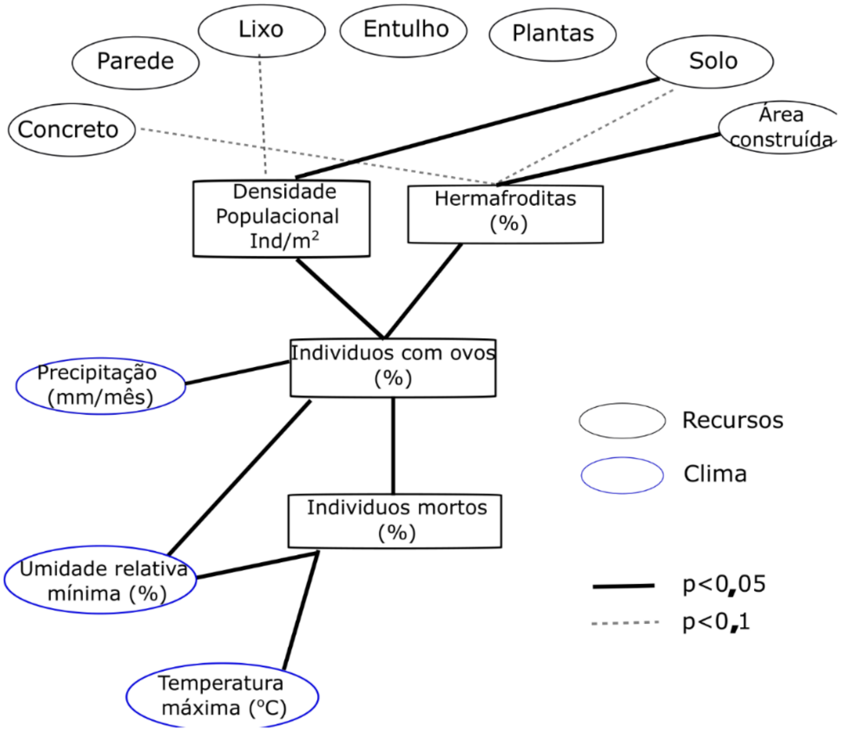

Figura 13. Associações entre a densidade populacional, porcentagem de indivíduos mortos, porcentagem de indivíduos com ovos, porcentagem de indivíduos hermafroditas, variáveis climáticas e variáveis de recursos na população de *Lissachatina fulica* do DF., segundo a significância baseada nos valores de p dos modelos lineares.

Agradecimentos.

Ao Instituto Brasília Ambiental (IBRAM) pela parceria estabelecida com o Laboratório de Biologia Evolutiva (2019-2022). A cada uma das pessoas nos 25 pontos de coleta que localizaram o caracol gigante africano e ajudaram em sua coleta. Aos voluntários do IBRAM em 2022 que acompanharam as coletas e o trabalho de laboratório. A Marina, Barbara e Gabriel pela ajuda nas coletas. A Thiago Nomiyama pelo acompanhamento e participação em: contato, marcação de visitas, transporte e coleta.

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CAPITULO 5. Shell shape as a potential predictor of age class in the invasive snail *Lissachatina fulica*

Paper accepted in December 2022 by Journal of Molluscan Studies

Marins, G., Patiño-Montoya, A., & Tidon, R. (2023). Shell shape as a potential predictor of age class in the invasive snail *Lissachatina fulica*. *Journal of Molluscan Studies, In press*.

Abstract

Understanding the life-history traits of invasive species can increase the effectiveness of eradication efforts and decrease costs. In the giant African snail *Lissachatina fulica*, one of the 100 worst invasive species on the planet, age structure can be an important indicator of invasiveness. Here, we associated variation in snail size with variation in weight and shell shape, in an effort to better understand the growth pattern in this species. Our study involved two components: we estimated the allometric relationship between weight and length in a natural population and monitored developmental variation in snail shape, weight, length and nutritional condition (condition factor) in a lab population. The weight and length growth patterns of the lab population were estimated independently using exponential regressions based on weekly measurements; shell shape was quantified using geometric morphometrics. In both populations, we found negative allometry between weight and length, suggesting that snail shells tend to elongate during growth. Geometric morphometrics confirmed this pattern and revealed that the change from a rounded to elongated shell shape does not occur at a constant pace but during a specific growth interval (i.e. at a shell length of 55–65 mm), which is described in the literature as the size when the animal becomes hermaphroditic. Future studies should test whether the shape change reported here corresponds to the transition to the hermaphrodite age class and, with this in mind, we provide two simple morphological indexes that allow us to characterize shell shape as rounded or elongated. If the shell size and shape indicate the transition to the hermaphroditism, our findings can help to place individuals of *L. fulica* into appropriate age classes and this will represent a useful baseline for development of eradication policies.

Keywords: giant African snail, *Achatina fulica*, invasion biology, age structure, geometric morphometrics.

Introduction

Due to its impacts, the giant African snail *Lissachatina fulica* (Bowdich, 1822) is recognized as one of the world's 100 worst invasive species (Lowe et al., 2000). This snail became invasive since its intentional introduction to India, from where it spread throughout Asia by 1930 and, due to human-mediated dispersal in the post-World War II era (i.e. mainly commercial trade), it reached most of the world's tropical areas (Raut & Barker, 2002). This mollusc can cause substantial economic losses because it feeds on crops such as lettuce, tomato, potato, coffee and apple (Raut & Barker, 2002; Thiengo et al., 2007). In areas of natural habitat, *L. fulica* may impact native malacofauna through competition and may disturb food webs by serving as prey for generalist predators (Colley & Fischer, 2009). With regard to public health, *L. fulica* can act as an intermediate host of pathogenic nematodes, such as *Angiostrongylus cantonensis* and *A. costaricensis*, which cause eosinophilic meningitis and abdominal angiostrongyliasis (Thiengo et al., 2007; Lv et al., 2009). For these reasons, in countries that lie in the invasive part of the range, such as Brazil, China, Colombia and the USA, *L. fulica* has attracted the attention of decision-makers as a species requiring control or eradication (MMA, 2006; Lv et al., 2011; MAVDT, 2011; USDA, 2012).

Knowledge of life-history traits in invasive species can support eradication efforts by decreasing costs and increasing effectiveness (Byers et al., 2002; Allendorf & Lundquist, 2003). For *L. fulica*, age structure can be an important indicator of invasiveness (Jayashankar, Sridhar & Verghese, 2013; Roda et al., 2016). Due to its protandrous hermaphroditism (i.e. the male reproductive system matures before the female reproductive system), *L. fulica* basically comprises three age classes: young, adult male and hermaphroditic adult (Tomiyama, 1993). Because the relative abundance of hermaphrodites is positively related to population fecundity rates, it is a good indicator of population growth and, consequently, invasiveness. Therefore, identifying hermaphrodites can improve plans for *L. fulica* management and eradication.

Despite efforts to assign the reproductive stages of *L. fulica* to specific size classes, a consensus has not been reached. Tomiyama (1993) related the presence of spermatocytes and oocytes to total shell length and found that hermaphroditic individuals had a mean shell length of 62 mm. In contrast, Roda et al. (2016) linked the presence of eggs to total shell length and showed that gravid individuals had a mean shell length of 75 mm. Additionally, Dickens, Capinera & Smith

(2018) raised snails in the lab from birth and observed that oviposition first occurred at an average shell length of 70 mm. These differences may reflect different environmental pressures, since growth endocrine regulation in individual snails is partly influenced by environmental factors, such as temperature, humidity, resource availability or quality (de Vaufleury, 2001; Rahman & Raut, 2010).

Shell shape can be a potential indicator of age classes or reproductive stages in *L. fulica*. A negative allometric weight–length relationship was found in Bahia, Brazil, where the growth rate for weight was slower than for shell length (Albuquerque et al., 2009). Weight–length allometric growth can simply be due to a condition change during growth, but it is often related to shape changes (Froese, 2006). In addition, shell shape analyses among isolated *L. fulica* populations has revealed wider variation at the intrapopulation scale than the interpopulation scale (Sobrepeña & Demayo, 2014a, 2014b), which could be partly due to shape changes during growth. Furthermore, protandrous hermaphroditism is known to reflect endocrine variation during the development of *L. fulica*. Androgens stimulate the development of male reproductive organs, while estrogens stimulate the development of female sexual organs (Takeda, 1985), and the relative proportions of these hormones could be the basis for morphological change. Therefore, it seems reasonable to speculate that variation in shell shape during the development of *L. fulica* could be related to differences in reproductive state.

The temporal variation in shells has stimulated a search for measurable morphological changes related to reproductive maturation, in an effort to assign snails to appropriate age classes. One example of a morphological parameter related to maturation in *L. fulica* is the thickness of the shell's peristome (lip), which varies between 0.5 and 0.8 mm for male adults and is higher than 0.8 for hermaphrodites (Tomiyama, 1993). However, using peristome thickness as the only parameter for age class discrimination may be susceptible to error since a 0.3 mm difference can be hard to detect using standard calipers. Therefore, it is important to search for additional methods to estimate age classes in *L. fulica*.

In this study, we aimed to explore the shell shape potential for age class determination in *L. fulica* by investigating how shell size, shell shape and weight of snails change during their development. We examined the relationship between snail weight, total shell length and shell shape for two samples: (1) a natural population collected in urban areas in the Brazilian Federal District and (2) a lab population that was monitored for 28 weeks. For this latter population, we also measured the nutritional condition of the snail using the condition factor (relationship between weight and length), which estimates the general wellbeing of these organisms on the assumption that at a given length heavier individuals are in better condition (Froese, 2006).

Material and Methods

Natural population

The field work took place between October 2019 and February 2020 in the Brazilian Federal District, which forms the core region of the Cerrado biome. In this region, this period corresponds to the rainy season when snail populations are expanded by the increase in snail activity after the aestivation period (dry season) and the consecutive recruitment of juveniles after successful breeding of the adult cohorts. We collected *Lissachatina fulica* using an opportunistic approach at locations indicated by the local environmental authority (Fig. 1). The individuals were collected manually and transported to the lab, where their weight and total shell length were measured. The measurement precision was 0.01 mm for length and 1 g for mass (except for neonates, 0.001 g).

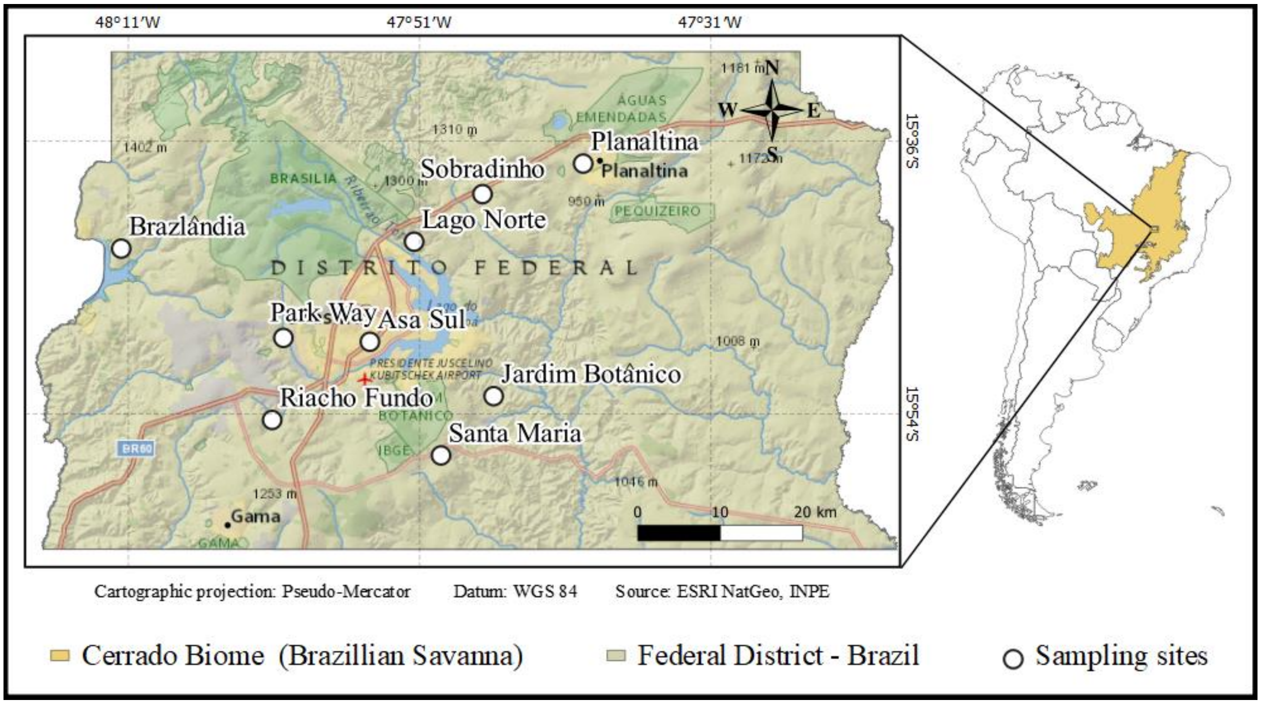

Figure 1. Sampling points of the giant African snail *Lissachatina fulica* in the Federal District, Brazil.

From the collected data, we calculated the allometric weight–length relationship (WLR), with the aim of (1) identifying the potential for shape modification during growth (since weight–length changes are usually related to shape changes) and (2) providing the information necessary to conduct a proper condition analysis, so its effect on allometry could be gauged. WLRs are a concept widely used by ichthyologists to predict the synchronic relationship of these variables and biologically interpret tendencies in its predictions (Froese, 2006). This relationship has also been used to evaluate the effect of climate variables on populations of *L. fulica* in Bahia, Brazil (Albuquerque et al., 2009), providing insights into snail conditions (condition factor). To assess the WLR, we used a regression based on the formula:

$$W = aLb$$

where W is weight, L is length, a is a constant linked with the general species shape and b is the rate at which weight changes with length (allometric coefficient) (Keys, 1928; Albuquerque et al., 2009). We then tested whether there was a significant difference between the allometric coefficient (b) and the isometric relationship (b = 3). For that, we used the hoCoef function from the Rpackage FSA (Ogle et al., 2021). All analyses were done in R v. 3.6.1 (R Core Team, 2020).

Lab population

For the lab experiment, 25 young adults (shell length 30–40 mm) (Tomiyama, 1993) of the snails collected in the field were individually raised in plastic containers (changed from 1.5 l to 4 l due to the growth of the individuals) with vermiculite substrate. The snails were fed weekly with a cabbage and carrot mixture, in addition to receiving 50 ml of water, and eggshells as a source of calcium. While the availability of food was correlated with the percentage increase in weight and total shell lengthg, we found that part of the food added in the previous week was still present in the container when we renewed it. The total shell length and weight of each snail were recorded weekly for 28 weeks, except for the interval between weeks 16 and 20, in which we accessed the individuals fortnightly due to the social isolation required by the COVID-19 pandemic. The weight and total shell length measurements were recorded with precisions of 0.001 g and 0.01 mm, respectively.

Using the weekly weight and total shell length averages of the lab population, we estimated the population growth pattern by performing exponential regressions. This type of regression was chosen due to data configuration and the possibility of analysing the weekly percentage of snails weight and length growth. The validity of the models was based on their assumptions and R^2 values. From the weight and length data, we calculated the variation in the snails' condition factor during their growth (Froese, 2006; Albuquerque et al., 2009). Since these measures increase at different rates, this factor seeks to compute snail conditions on the basis of a ratio that balances the importance of weight and length (Froese, 2006). Variation in snail conditions can affect the WLR results and inferences, that is a continuous decrease in the snails' nutritional condition through growth could generate the allometry between weight and length rather than a change in shape (Froese, 2006). Hence, the analysis of conditions during growth enhances the interpretive power of the results and was used here as background to interpret and discuss them. To avoid distortions, the condition factor (K) was calculated from the formula $K = W/L^b$ using the parameter b (allometric coefficient) of the WLR of the wild population (Lima-Junior, Cardone & Goitein, 2002; Albuquerque et al., 2009).

To measure ontogenetic shape variation, we monitored the 25 individuals kept in the lab as shell length increased from 45 to 90 mm; snails are supposed to reach the hermaphrodite age class during this interval (Tomiyama, 1993; Roda et al., 2016; Dickens et al., 2018). The individuals were initially photographed every 3 weeks and then fortnightly. The shells were positioned on white paper with the apertural view facing the camera and fixed with tape to maintain the correct position. We used a 10 cm ruler as the scale bar and imaging was done with a Canon EOS Rebel T6 digital camera on a fixed tripod. Twelve landmarks were digitized on each snail photo (Fig. 2), and their cartesian coordinates were saved using the software tpsDIG v. 2.25 (Rohlf, 2013). These coordinates were then submitted to Procrustes analysis with the software MorphoJ 1.07[a] (Klingenberg, 2011) to remove the effects of rotation, position, orientation and size variation (Rohlf & Slice, 1990); the shape change vector values were imported into MorphoJ to visualize the change in shape. We then performed a principal component analysis (PCA) and used partial redundancy analysis (RDA) to evaluate how much of the shape variation was explained by time with individuals as a covariate. To avoid bias arising from the number of independent variables and sample size, we adjusted the R^2 of the RDA (Borcard, Gillet & Legendre, 2018). To evaluate the significance of the RDA model, we performed a permutation analysis of variance.

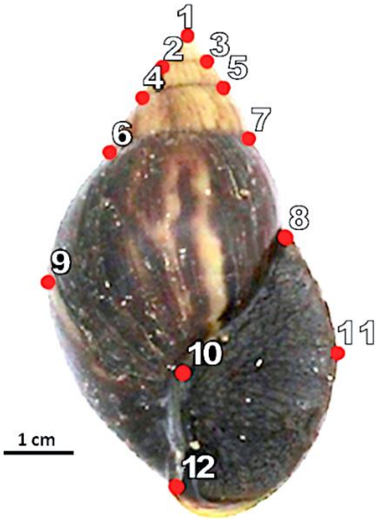

Figure 2. Shell of the giant African snail *Lissachatina fulica*, showing ten landmarks (1, 2, 3, 4, 5, 6, 7, 8, 10, 12) and two pseudolandmarks (9, 11) used to capture shell size and shape.

Results

Natural population

We collected a total of 503 individuals of *Lissachatina fulica*, varying from 0.03 to 103 g in weight (W) (54% of them weighed less than 10 g). Total shell length (L) varied from 4.20 to 101.85 mm and the most frequent interval was 30–40 mm (23% of individuals). While the frequency distribution for mass shows that most individuals are in the lower mass classes, length is distributed more evenly (Fig. 3). The calculated weight–length relationship was $W = 0.0006 L^{2.5762}$ ($R^2 = 0.988$; df = 501; $P < 0.001$) (Fig. 3). We rejected the null hypothesis that the allometric coefficient (b) was 3.0 ($t = -33.9$; df = 501; $P < 0.001$), with b lying between 2.55 and 2.60 (95% confidence interval). This shows that this *L. fulica* population is characterized by negative allometric growth.

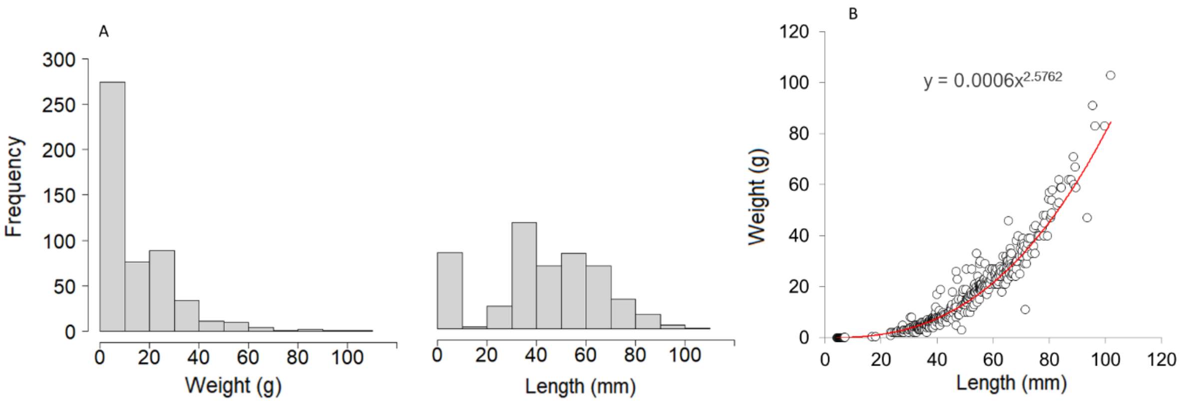

Figure 3. Weight and length of a natural population of *Lissachatina fulica*. A) Frequency distributions. B) Allometric relationships. Based on 503 individuals collected at the Federal District, Brazil, from October 2019 to February 2020.

Lab population

The mean weight increased from 7.177 ± 0.220 to 54.686 ± 2.162 g over the 28 weeks of the experiment, that is a c. 662% increase in weight. The exponential model had all the assumptions accepted and a high R^2 (0.987). It represents a positive exponential curve in which y = 7.5525e0.0706x (R^2 = 0.987; df = 24; $P < 0.001$); the *L. fulica* weight was multiplied by $e^{0.0706}$, approximately 1.073 every week, which means that the snail grew 7.3% in weight each week. The mean total shell length varied from 39.13 ± 0.46 to 77.98 ± 1.14 mm over the 28 weeks (i.e. it practically doubled). The exponential model was the closest to normality and homoscedasticity, in addition to having the highest R^2 value (0.988). This model, $y = 38.042e^{0.0254x}$ (R^2 = 0.988; df= 24; $P < 0.001$), represents a smoother positive exponential curve than that observed for weight and reveals that *L. fulica* total shell length is multiplied by $e^{0.0254}$, that is by c. 1.026g every week, which means that the snail grows 2.6% in length each week. The mean condition factor (K) when the snails arrived at the lab was 5.77 ± 0.07, which was the lowest throughout the experiment. One week later, K increased to 6.98 ± 0.12, suggesting that lab conditions were better than natural conditions. During the experiment, K remained relatively stable, oscillating from 6.47 ± 0.10 to 7.71 ± 0.10 with high increases followed by low consecutive decreases, suggesting that snails invested primarily in increasing weight and, secondarily, in increasing in length, a pattern that was weaker after the week 20 (Fig. 4), when snails averaged 60 mm and had already undergone the maturity shape change (Fig. 5).

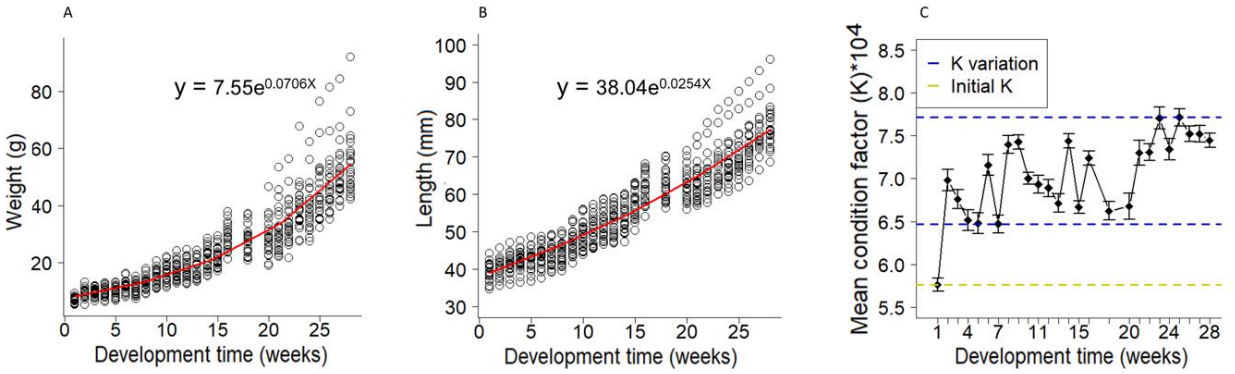

Figure 4. Weight (A) and length (B) growth patterns of *Lissachatina fulica* in a population maintained in the laboratory for 28 weeks. C) Variation in the mean condition factor (K) of the same population multiplied by 10^4 for better visualization. The bars represent the mean standard error.

The PCA and RDA revealed shell shape variation over time, indicating an allometric effect (Fig. 5). Intrapopulation shape variation was higher in weeks 10 and 13 due to the dispersed PC1 values. In the subsequent weeks, PC1 stabilized close to its median and PC2 changed from negative to mostly positive values. According to the RDA, development time explained 26.5% of the shape variation when variation between individuals was kept constant ($R^2 = 0.196$; df = 7; $P < 0.001$). Hence, we refer to this variation when talking about ontogenetic shape variation. The first canonical axis (RDA1) explained 51.97% of the ontogenetic shape variation and was primarily associated with landmarks 7, 8, 9, 10 and 12. Higher RDA1 scores corresponded to wider and shorter individuals, with smaller distances between the first suture and shell aperture (landmark 7 to 8, represented in blue and by the letter ‘b’ in Fig. 6) and between the columella plait and columella truncation (landmark 10 to 12, represented in orange and by the letter ‘c’ in Fig. 6) with respect to narrower and longer individuals. In contrast, lower RDA1 values corresponded to more elongated and narrow individuals, with larger distances between landmarks 7–8 and 10–12. In sum, when the snails reached a length of 55–65 mm (i.e. between weeks 13 and 16, their shape changed abruptly (Fig. 5), transforming from a shorter shape to a more elongated one.

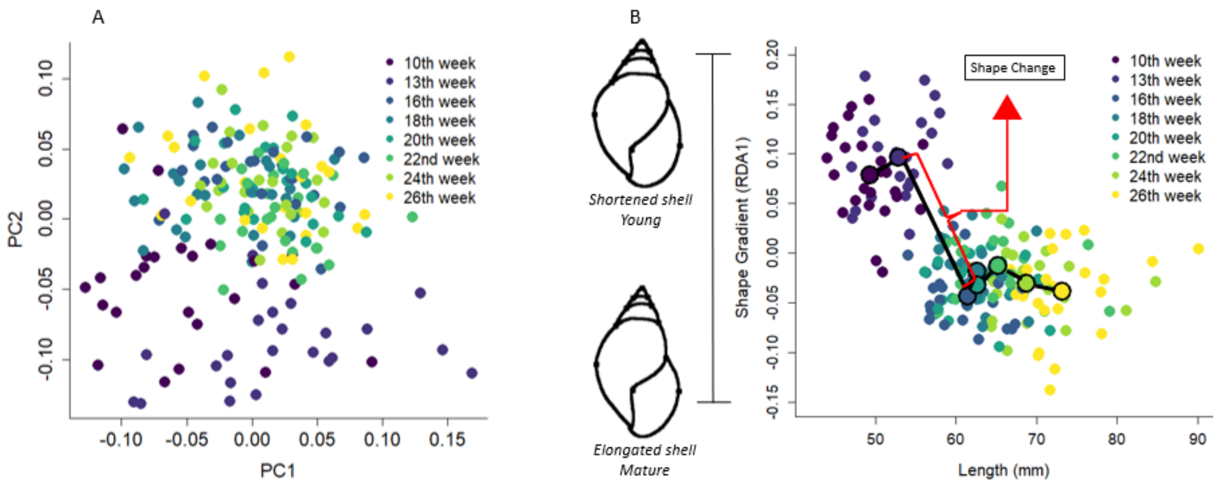

Figure 5. Ontogenetic shape changes of *Achatina fulica* shell in a popularion cultivated over 28 weeks in the laboratory. A) Principal component analysis (PCA) of the Procrustes coordinates. PC1 and PC2 disregard the variation explained by intrapopulational variation (25.9%). B) Redundancy analysis (RDA) showing shape variation related to length and time. The shells on the left illustrate the gradient of form represented in the graph, and the colors represent different time points.

Discussion

In this study, we showed that *Lissachatina fulica* may show an allometric growth pattern for weight and shell length and shape. Shell shape becomes more elongated during development, but this change does not occur at a constant pace. A relatively abrupt change occurs when snails reached a length of 55–65 mm and are supposedly experiencing the transition to hermaphroditism. In what follows, we discuss these findings and suggest future avenues for research.

The weight–length allometric relationship in the natural population studied here grees with results obtained in the metropolitan region of Salvador (Bahia State), a city located c. 1080 km from Brasilia (Albuquerque et al., 2009). The value of the parameter a was the same (a = 0.0006), indicating that both *L. fulica* populations share similar general shell shapes (Keys, 1928) and suggesting the possibility that other populations may have similar values. Similarly, parameter b was c. 2.6 in both populations, suggesting negative allometric growth for weight–length in *L. fulica*. The growth patterns calculated from the lab population revealed that the rate of increase in weight was 2.81 times higher than the corresponding rate in total shell length growth and not the threefold increase expected for isometric growth (Keys, 1928; Froese, 2006). Therefore, even under lab-controlled conditions, where resources are abundant, *L. fulica* showed negative weight–length allometric growth. The condition factor could be interpreted as individual health or individual shape (Froese, 2006). In this study, the condition factor did not decrease in older individuals; thus, we suggest that the allometry between weight and total shell length is explained by shell shape (Froese, 2006).

The geometric morphometrics showed that shell shape did change through growth, representing 26.5% of the analysed variation in shape; this largely involved changes in the width and the distance between the shell aperture and first suture and between the columella plait and columella truncation. Furthermore, the shape change in *L. fulica* occurred abruptly, 13 to 16 weeks after the beginning of the experiment (which represents the stage when young adults become hermaphrodites) when the shell is between 55–65 mm in length. This is consistent with size estimates for the male–hermaphrodite transition (Tomiyama, 1993; Roda et al., 2016; Dickens et al., 2018). This morphological change could be triggered by endocrine variation related to snail sexual development, since the transition to hermaphroditism happens consonantly with the increase in oestrogens in the snail system (Takeda, 1985). A study performed with a marine-littoral snail showed that differences in growth rate led to shell morphological variation similar to that observed in our study (Kemp & Bertness, 1984). Kemp & Bertness (1984) revealed that snails with higher growth rates (as is expected for young individuals) have a more globular shell shape than snails with lower growth rates. Hence, reproductive hormonal variation can be the foundation for this allometry by decreasing the growth rate in fully reproductive individuals.

Shape analysis combined with measurements of peristome thickness (Tomiyama, 1993) can potentially contribute to determining age classes in *L. fulica* populations and thus help to advance eradication efforts. Shell shape and peristome thickness unlike shell length, seem to be related to endocrine variation that occurs during reproductive maturation. Together, these two morphological characters could provide the certainty needed for the applicability of eradication based on population invasiveness, represented by the frequency of hermaphrodites (Jayashankar et al., 2013; Roda et al., 2016). As noted by Colley & Fischer (2009), for the 11 monitored sites in Guaraqueçaba, Brazil from which *L. fulica* was eradicated recolonization was 95.7% after 5 months. The recolonization probability is linked to the frequency of egg-laying hermaphrodites since *L. fulica* oviposit underground, where hatchlings can remain for 15 days (Raut & Barker, 2002). Despite the high recolonization rate, the size of the collected individuals decayed almost 30% on the second sampling (Colley & Fischer, 2009). If all collected snails had been separated into age classes, we would probably observe a drastic decay in the frequency of hermaphrodites in the population. Analysing the frequency of hermaphrodites during eradication programmes can show the probability of recolonization for different sites and help decision-makers plan for future actions.

As we did not dissect the snails or perform an endocrine evaluation, we cannot say if the subset of snails with more elongated shells had more eggs or higher levels of oestrogen than the subset of snails with rounded shells. This hypothesis, however, can be easily tested in the future if we convert the 26.5% of ontogenetic shape variation into an easy-to-use morphological index. Since the RDA is a constrained ordination with orthogonal axes, it is expected that the shape changes explained by development occur independently of the variation explained by different variables (i.e. environmental variables). Therefore, we propose two shape indexes that account for most of the shape differences observed between young and old individuals (i.e. they were based on the distances most important for shape variation, as calculated by the RDA; Fig. 6). The first shape index reflects how many times the distance between the first suture and the shell aperture (landmarks 7 to 8) fits into the shell width (landmarks 8 to 9). The second index measures how many times the distance between the columella plait and the columella truncation (landmarks 10 to 12) fits into the shell width. Before the abrupt change in shell shape, both indexes averaged c. 2.50, while after the shape change, they decreased to approximately 2.1 (these values differ from Fig. 6 because these are mean values for each group; Fig. 6 shows extreme values, which are based on the shape axis of Fig. 5). Since these shape changes seem to be linked to reproductive maturation, we suggest that future work test whether these morphological indexes are linked with characters of the internal reproductive anatomy. If so, this approach might aid the identification of age classes in populations and thereby contribute to control efforts.

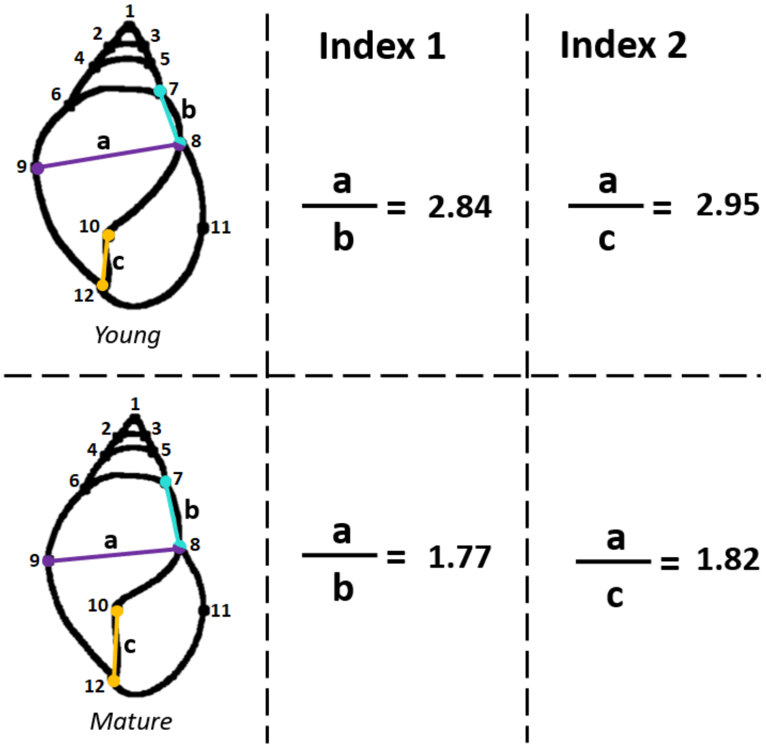

Figure 6. Shape indexes proposed to classify young and mature snail shells. a: Shell width (landmark 8 to 9), b: distance between the first suture and the shell lip (landmark 7 to 8), c: distance between columella plait and the columella truncation (landmarks 10 to 12).

Acknowledgements

We are grateful to Anandha de Almeida Silva for her valuable help in collecting data and to Universidade de Brasília and Instituto Brasília Ambiental for providing the infrastructure necessary for this study. We also thank Dr John Grahame, Dr Dinarzarde Raheem and two anonymous referees for their careful reading of the manuscript and the many insightful comments and suggestions. This work was supported by the Coordenação de Pessoal de Nível Superior (code 001), Conselho Nacional de Desenvolvimento Científico e Tecnológico and Fundação de Apoio a Pesquisa do Distrito Federal.

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Conclusões finais

Esta tese estudou a resposta do caracol gigante africano *Lissachatina fulica* ao clima em diferentes escalas e na presença de recursos. Para estudar a resposta de *L. fulica* é necessário o conhecimento dos atributos que caracterizam seu potencial invasor e as características dos locais onde ocorre. Portanto, para avaliar a resposta ao clima em escala global esta tese usou a ocorrência. Para avaliar a resposta ao clima em escala regional esta tese usou a densidade populacional. Para avaliar a resposta ao clima em escala local e na presença de recursos, esta tese usou a densidade populacional, porcentagem de indivíduos mortos, porcentagem de indivíduos com ovos e porcentagem de indivíduos hermafroditas.

A revisão sistemática mostrou que o conhecimento sobre o caracol gigante africano e os locais onde ocorre está fragmentado e limitado. Já que, os dados básicos para conhecimento apresentam dilemas. Por exemplo, os estudos de distribuição potencial de *L. fulica* publicados até 2020 mostram uma grande quantidade de registros de ocorrência em diferentes intervalos de tempo, no entanto, também mostram localidades com baixa suceptibilidade do molusco, onde realmente ocorre. A abundância e a densidade populacional são os dados mais registrados, ainda assim, estão sem uma clara direção na sua variação temporal e espacial, o que é necessario para estabelecer o umbral de impacto. E, nos aspectos reprodutivos, a determinação das classes etárias apresentam diferenças dentro da literatura. Assim, quando se está pensando em controle de *L. fulica* fica difícil saber se realmente a espécie está em um ambiente semelhante da área nativa (ou adequável), se as densidades registradas representam algum impacto significativo ou quando pode ocorrer uma recolonização.

A resposta de *L. fulica* ante a variação climática foi avaliada em escala global, regional e local. Em escala global, as diferenças temporais nas introduções entre as regiões biogeográficas influenciaram o nicho climático realizado atual de *L. fulica.* Especialmente, a região Neotropical apresentou a maior expansão em comparação com a área nativa, talvez, por ser a ultima região onde se estabeleceu o molusco. Em escala regional, os resultados apontam que *L. fulica* no Neotropico mantem baixas densidades populacionais em qualquer combinação climática, e as densidades podem incrementar com combinações especificas. No entanto, em escala local a densidade populacional não respondeu com a variação climática, talvez, pelos baixos valores de densidade populacional (<1 ind/m^2) de *L. fulica* no Distrito Federal. A porcentagem de indivíduos mortos e a porcentagem de indivíduos com ovos sim responderam com a variação climática local. Estes resultados reforçam que *L. fulica* é uma espécie com uma ampla tolerância climática e chama atenção sobre o tipo de resposta segundo a escala espacial e temporal do estudo.

A resposta de *L. fulica* ante a presença ou ausença de recursos foi avaliada em escala local e diferiu da resposta ante variação climática. A densidade populacional respondeu com a presença de solo e marginalmente significante com a presença de lixo. A porcentagem de indivíduos hermafroditas respondeu com a porcentagem de área construída. Estes resultados coincidem parcialmente com a associação de *L. fulica* ao homem e as áreas urbanas. Além disso, estes resultados chamam atenção sobre o conhecimento da dinâmica local de *L. fulica* para seu manejo e não estabelecer estratégias com conhecimento de outros locais.

No capitulo 1 foi estabelecida a dificuldade existente na separação dos indivíduos jovens, machos e hermafroditas em *L. fulica*, e no capitulo 4 os indivíduos coletados tiveram que ser dissecados para estabelecer o comprimento mínimo com ovos. O capitulo 5 contribue com a proposta de dois índices para identificar aos indivíduos hermafroditas. Os indivíduos hermafroditas são aqueles que colocam ovos e, portanto, participam ativamente no mantenimento das populações. Além disso, as analises morfometricas demonstram que o fator de condição derivado da equação da curva de peso vs. comprimento pode ser analisado tanto como qualidade do individuo como um produto de mudança na forma durante o crescimento. Com estes resultados estamos mais próximos de melhorar a eficiência das estratégias de manejo do caracol gigante africano.

Por fim, este documento abre a porta para o desenvolvimento de múltiplas linhas de geração de conhecimento sobre as populações não nativas de *L. fulica*, sendo possível otimizar as estratégias de manejo. Uma linha pode ser encaminhada entorno da mudança do nicho climático realizado de *L. fulica* com as recentes introduções na Europa ou com as mudanças climáticas. Outra linha pode ser sobre a relação entre a densidade populacional e o impacto (ecológico, saúde, económico). Além disso, não podemos esquecer os estudos de história de vida da espécie, já que parece que seus atributos apresentam uma ampla variação entre as localidades não nativas.

Material suplementar

Capitulo 2:
https://onlinelibrary.wiley.com/doi/abs/10.1111/ivb.12385

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