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Exploring the invasion dynamics and impacts of the invasive Asian common toad in Madagascar

Fulvio Licata

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Exploring the invasion dynamics and impacts of the invasive Asian common toad in Madagascar

Fulvio Licata

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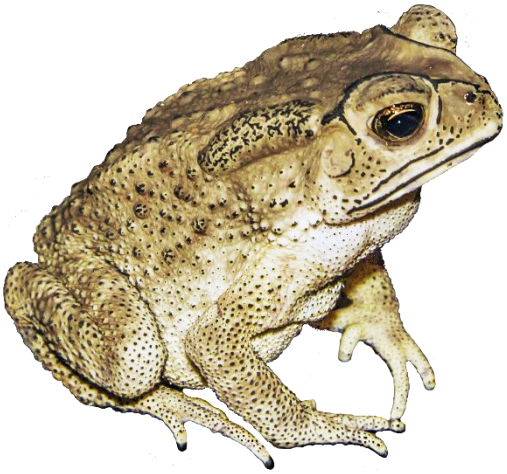

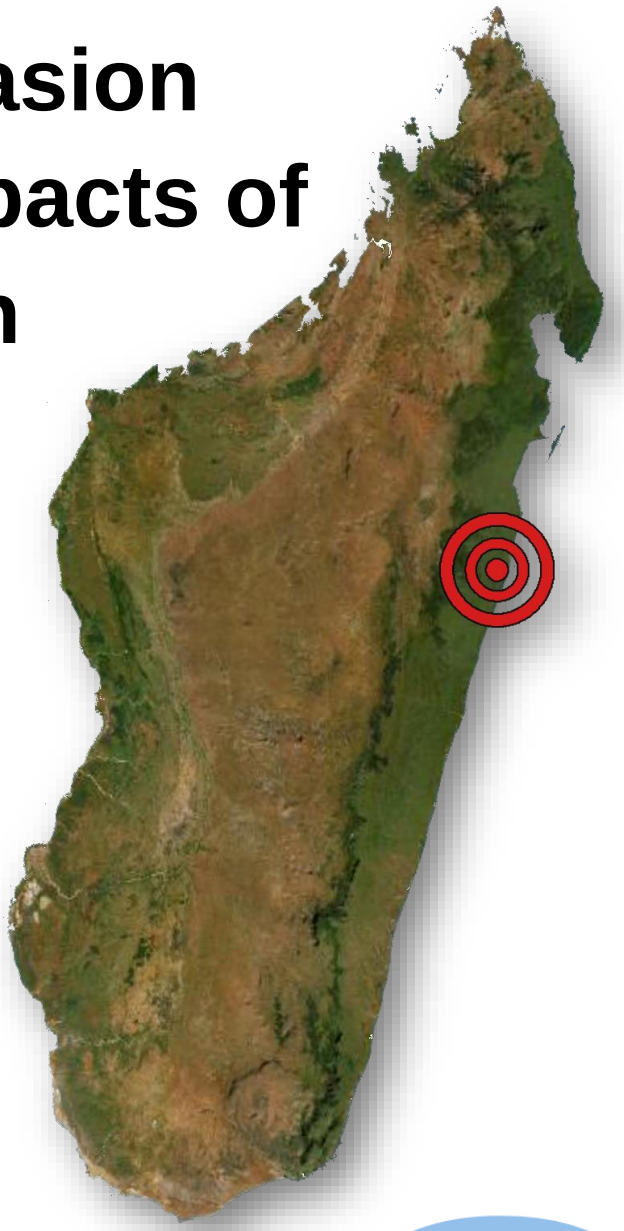

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Nota prévia

Na elaboração desta tese, e nos termos do número 2 do Artigo 4º do Regulamento Geral dos Terceiros Ciclos de Estudos da Universidade do Porto e do Artigo 31º do D.L. 74/2006, de 24 de Março, com a nova redação introduzida pelo D.L. 230/2009, de 14 de Setembro, foi efetuado o aproveitamento total de um conjunto coerente de trabalhos de investigação já publicados em revistas internacionais indexadas e com arbitragem científica, os quais integram alguns dos capítulos da presente tese. Tendo em conta que os referidos trabalhos foram realizados com a colaboração de outros autores, o candidato esclarece que, em todos eles, participou ativamente na sua conceção, na obtenção, análise e discussão de resultados, bem como na elaboração da sua forma publicada. Este trabalho foi financiado pela Fundação para a Ciência e Tecnologia (FCT, Portugal) através de um Exploratory Reserch Project (IF/00209/2014/CP1256/CT0011) atribuído a Angelica Crottini, através de uma bolsa de Doutoramento SFRH/BD/131722/2017 atribuída ao candidato Fulvio Licata, e através dos contratos de Investigador FCT (IF/00209/2014) e 2020.00823.CEECIND atribuídos a Angelica Crottini. O Capítulo 5 foi parcialmente financiado pela Saint Louis Zoo's Field Research for Conservation program of the Wildcare Institute através da bolsa FRC# 2018.01 atribuída ao candidato.

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Resumo

As espécies exóticas invasoras (IAS) são um dos principais impulsionadores de alteração global, com impactos severos nos ecossistemas e sociedades. As biotas insulares e os hotspots de biodiversidade podem ser particularmente vulneráveis a invasões biológicas, especialmente em países com baixa capacidade de combater as IAS. As invasões de anfíbios podem ter impactos importantes nos ambientes receptores, incluindo competição, predação, transmissão de doenças, entre outras, no entanto, poucas espécies têm sido extensivamente estudadas. Investigar a dinâmica de invasão e os impactos de anfíbios invasores pouco estudados em países em desenvolvimento pode ser relevante para melhorar a nossa compreensão e gerir a problemática das invasões de anfíbios em todo o mundo.

O sapo comum asiático *Duttaphrynus melanostictus* é nativo do sudeste da Ásia, é altamente fecundo, tóxico e pode representar um vetor de doenças de anfíbios. Em 2014, uma população invasora dessa espécie foi relatada em Madagáscar, levantando preocupações sobre os possíveis impactos nos seus ecossistemas megadiversos. Neste estudo, exploro a dinâmica de invasão e os impactos do sapo comum asiático em Madagáscar através de um conjunto de estudos que visam examinar: (1) a distribuição e taxa de propagação, (2) os fatores que regulam a abundância de sapos, (3) morfologia, (4) ecologia espacial, (5) a ocorrência e magnitude do efeito tóxico sobre predadores nativos, (6) e os potenciais impactos socioeconómicos sobre as populações locais.

Inicialmente, avaliei o alcance invasivo e a taxa de disseminação da invasão usando registos de distribuição. Além disso, avaliei também os fatores ambientais que determinam a abundância de sapos em seis localidades em áreas urbanas e rurais em toda a área de invasão. Demonstro que o alcance invasivo aumentou cinco vezes ao longo de três anos, indicando uma aceleração da invasão. As abundâncias de sapos foram heterogéneas, porém as densidades foram maiores na presença de resíduos orgânicos. Estes resultados confirmaram o alto potencial invasor dessa espécie, sugerindo que programas adequados de controlo e descarte de resíduos podem ajudar a reduzir a abundância de sapos.

Testei também se a expansão acelerada do alcance poderia ser determinada pela classificação espacial de características relevantes para a dispersão, conduzindo uma análise morfológica de sapos adultos capturados em várias localidades ao longo do gradiente de invasão. Não encontrei nenhuma evidência de ordenação espacial nas características analisadas. Pelo contrário, descobri que a variação morfológica é amplamente dependente do sexo e do tamanho corporal dos indivíduos. Além disso, o tamanho dos sapos não variou ao longo da área de invasão, o que pode indicar que adultos e juvenis contribuem para a expansão da área de distribuição da espécie. Esta hipótese também foi apoiada por observações de campo de juvenis superando os adultos na frente de invasão. Este trabalho fornece pela primeira vez informação sobre a dinâmica

de invasão desta espécie em Madagáscar, destacando diferenças importantes com a invasão do sapo-cururu na Austrália.

Para entender os fatores que determinam a dinâmica de propagação, conduzi um estudo de rastreamento de rádio de sapos adultos em três localidades ao longo do gradiente de invasão, testando a ocorrência de ordenação espacial de traços comportamentais e investigando determinantes intrínsecos e extrínsecos do comportamento espacial. Os sapos apresentaram comportamento sedentário e os seus movimentos foram ditados pelas condições climáticas. A classificação espacial do comportamento de dispersão não foi detectada, e a distribuição de frequência dos movimentos não foi exponencialmente limitada, suportando um cenário de invasão dominado por dispersão de ponta de curta distância, com expectativa de que a velocidade de invasão aumente ao longo do tempo.

O envenenamento por sapos de predadores nativos é o impacto mais temido da invasão de sapos asiáticos em Madagáscar devido à alta vulnerabilidade prevista dos predadores nativos às suas toxinas. A magnitude desses impactos provavelmente depende da capacidade dos sapos de colonizar habitats florestais, onde predadores comedores de rãs são especialmente abundantes. Depois de confirmar a capacidade dos sapos de invadir florestas, documentei envenenamento letal por sapos na cobra nativa *Madagascarophis colubrinus*. Estimativas mensais de mortalidade de cobras devido ao envenenamento por sapos demostraram que, se constante ao longo do tempo, esse impacto poderia reduzir pela metade a população de predadores em um ano. Além de destacar o impacto potencialmente disruptivo dos sapos em uma cobra nativa, este estudo também sinaliza a necessidade de estudos futuros que se concentrem nas possíveis repercussões do declínio de predadores induzido por sapos na saúde humana devido à facilitação indireta de pragas de roedores comensais humanos.

Por fim, usando dados de questionários publicos, juntamente com uma abordagem multianalítica, demonstrei que: (1) os locais identificam as espécies de forma confiável, (2) a invasão seguiu uma expansão linear de aprox. 2 km / ano, embora a dispersão mediada pelo homem tenha facilitado a disseminação da espécie a longa distância, (3) a percepção pública negativa é maior nas áreas rurais (devido aos altos impactos observados nos ecossistemas) e em localidades recentemente invadidas, sugerindo efeitos dependentes da densidade ou do tempo; perda de apiários domésticos, envenenamento de aves e declínio de serpentes foram os impactos que causaram as maiores preocupações da sociedade. Com este estudo, descrevo as complexas interações homem-sapo em diferentes contextos ambientais e em diferentes estágios da invasão. Destaquei a extrema versatilidade dos dados obtidos através de questionários públicos para abordar as questões fundamentais da ciência das invasões e implementar medidas de gestão, que podem ser especialmente úteis em regiões do mundo de difícil monitorização com baixa capacidade para combater IAS.

Esta tese fornece informações de base importantes para a gestão do sapo asiático invasor tanto em Madagáscar como em outros locais, destacando a importância de investigar aspectos fundamentais da biologia do IAS para entender os mecanismos subjacentes à dinâmica e aos impactos da invasão. Em resumo, demonstrei que a expansão da área de distribuição é linear e relativamente lenta, embora a dispersão mediada pelo homem facilite a propagação da espécie a longa distância, enfatizando a importância de implementar medidas de biossegurança, bem como desenvolver sistemas de detecção precoce e resposta rápida. A invasão de sapos em habitats florestais e o envenenamento letal de um predador comedor de rãs podem potencialmente determinar extinções locais de algumas espécies nativas, com potenciais cascatas tróficas em ecossistemas e possíveis implicações também para a saúde humana. Além disso, uma combinação de impactos potenciais nas comunidades locais, se confirmadas, pode aprofundar as vulnerabilidades socioeconómicas sistémicas em Madagáscar.
Mais amplamente, este trabalho fornece informação importante sobre os desafios associados à gestão de espécies invasoras altamente problemáticas em países em desenvolvimento, oferecendo sugestões metodológicas para o estudo da dinâmica de invasão e impactos que são transferíveis para outros sistemas de estudo.

Palavras-chave

Duttaphrynus melanostictus, espécies exóticas invasoras, invasões biológicas, hotspot de biodiversidade, modelos *N*-mixture, rádio telemetria, questionários públicos, modelos de ocupação com falsos positivos, modelos lineares mistos, abundância da espécie, ecologia espacial, impactos socioeconômicos, impactos ecológicos

Abstract

Invasive alien species (IAS) are a major driver of global change, having severe impacts on ecosystems and societies. Insular biotas and biodiversity hotspots can be particularly vulnerable to biological invasions, especially within countries with a low capacity to counter IAS. Amphibian invasions can have important impacts on recipient environments, including competition, predation, and transmission of diseases, among others, however, only a few species have been extensively studied. Investigating the invasion dynamics and impacts of understudied invasive amphibians in biodiverse developing countries can be relevant to improving our understanding and management of amphibian invasions worldwide.

The Asian common toad *Duttaphrynus melanostictus* is native to southeast Asia, is highly fecund, toxic, and could represent a vector for amphibian diseases. In 2014, an invasive population of this species has been reported in Madagascar, raising concerns about the potential impacts it could have on its megadiverse ecosystems. In this study, I explore the invasion dynamics and impacts of the Asian common toad in Madagascar through a set of studies that examine: (1) the distribution and rate of spread, (2) the factors regulating toad abundances, (3) morphology, (4) spatial ecology, (5) the occurrence and magnitude of toxic effect on native predators, (6) and the potential socioeconomic impacts on local populations.

Initially, I assessed the invasive range and the rate of spread of the invasion using distributional records. Furthermore, I evaluated the environmental factors determining toad abundances in six localities across urban and rural areas across the invasive range. I demonstrated that the invasive range increased fivefold over three years, showing an acceleration of the invasion. Toads' abundances were heterogeneous, but densities were highest in the presence of organic waste. These results confirmed the high invasive potential of this species, suggesting that proper waste control and disposal programs could help reduce toad abundance.

I tested whether the accelerating range expansion could be caused by spatial sorting of dispersal-relevant traits, conducting a morphological analysis of adult toads captured in several localities along the invasion gradient. I found no evidence of spatial sorting in the traits analyzed. On the contrary, I found that morphological variation was largely dependent on the sex and body size of individuals. Furthermore, toads' size did not vary across the invasive range, which could indicate that both adults and juveniles contribute to the range expansion of the species. This hypothesis was also supported by field observations of juveniles outnumbering adults at the invasion front. This work provides the first insights into the invasion dynamics of this species in Madagascar, highlighting important differences with the Cane toad invasion of Australia.

To understand the factors determining the spreading dynamics, I conducted a radio-tracking study of adult toads in three localities along the invasion gradient, testing the occurrence of spatial sorting of behavioral traits, and investigating intrinsic and extrinsic determinants of spatial behavior.

Toads showed sedentary behavior, and their movements were dictated by weather conditions. Spatial sorting of dispersal behavior was not detected, and the frequency distribution of movements was not exponentially bounded, supporting an invasion scenario dominated by short-distance leading-edge dispersal, with invasion speed expected to increase over time.

Toad poisoning of native predators was the most dreaded impact of the Asian toad invasion to Madagascar due to the predicted high vulnerability of native predators to its toxins. The magnitude of these impacts likely depends on the ability of toads to colonize forest habitats, where frog-eating predators are especially abundant. After confirming toads' capability of invading forests, I documented lethal toad poisoning in the native cat-eyed snake *Madagascarophis colubrinus*. Monthly estimates of snakes' mortality due to toad poisoning showed that, if constant through time, this impact could halve the predator population within one year. Besides highlighting the potentially disruptive impact of toads on a native snake, this study also urges future research to focus on the potential repercussions of the toad-induced decline of predators on human health due to indirect facilitation of human-commensal rodent pests.

Lastly, using public survey data, coupled with a multi-analytical approach, I showed that: (1) locals reliably identify the species, (2) the invasion followed a linear range expansion of approx. 2 km/year, although man-mediated dispersal was found to facilitate the long-distance spread of the species, (3) negative public perception is highest in rural areas (due to high perceived impacts on ecosystems), and in recently invaded localities, suggesting density- or time-dependent effects; loss of domestic apiaries, poisoning of poultry, and decline of snakes were the impacts that caused the greatest societal concerns. With this study, I describe the complex human-toad interactions in different environmental contexts and at different stages of the invasion. I highlighted the extreme versatility of public survey data to address fundamental requirements for invasion science and management applications, which can be especially useful in hard-to-monitor regions of the world with a low in-country capacity to counter IAS.

This thesis provides important baseline information for the management of the invasive Asian toad both in Madagascar and elsewhere, highlighting the importance of investigating fundamental aspects of IAS biology to understand the mechanisms underlying invasion dynamics and impacts. In summary, I showed that the range expansion is linear and relatively slow, although man-mediated dispersal facilitates the long-distance spread of the species, stressing the importance of implementing biosecurity measures, as well as developing early detection and rapid response systems. Toad invasion of forest habitats and lethal poisoning of a frog-eating predator can potentially determine local extinctions of some native species, with potential trophic cascades across ecosystems, and possible implications also for human health. In addition, a combination of potential impacts on local communities, if confirmed, can deepen systemic socioeconomic vulnerabilities in Madagascar.

More broadly, this work provides important insights into the challenges associated with the management of highly problematic invasive species in developing countries, offering methodological suggestions for the study of the invasion dynamics and impacts which are transferable to other study systems.

Keywords

Duttaphrynus melanostictus, invasive alien species, biological invasions, biodiversity hotspot, *N*-mixture models, radiotelemetry, public surveys, false-positive occupancy models, linear mixed models, species abundances, spatial ecology, socioeconomic impacts, ecological impacts

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1. General introduction

1.1 Biological invasions

The weakening of biogeographical dispersal barriers is one of the major consequences of human activities and is causing unprecedented translocations of organisms beyond their native range, where they are defined as 'alien species' (Essl et al. 2018). This phenomenon became accentuated during the past two centuries with increasing globalization patterns (Seebens et al. 2021b) and shows no signs of slowing down (Seebens et al. 2017, 2021a). Human-mediated introduction of alien species may occur both intentionally and accidentally (e.g., via accidental stowaways or escape) (Hulme 2009). In the case of human-mediated pathways, alien species must overcome several barriers before becoming invasive; this is known as the invasion process, and initiates when a species is transported to a new area, where it survives, establishes a self-sustaining population, proliferates, and starts spreading to new habitats (Fig. 1.1; Blackburn et al. 2011).

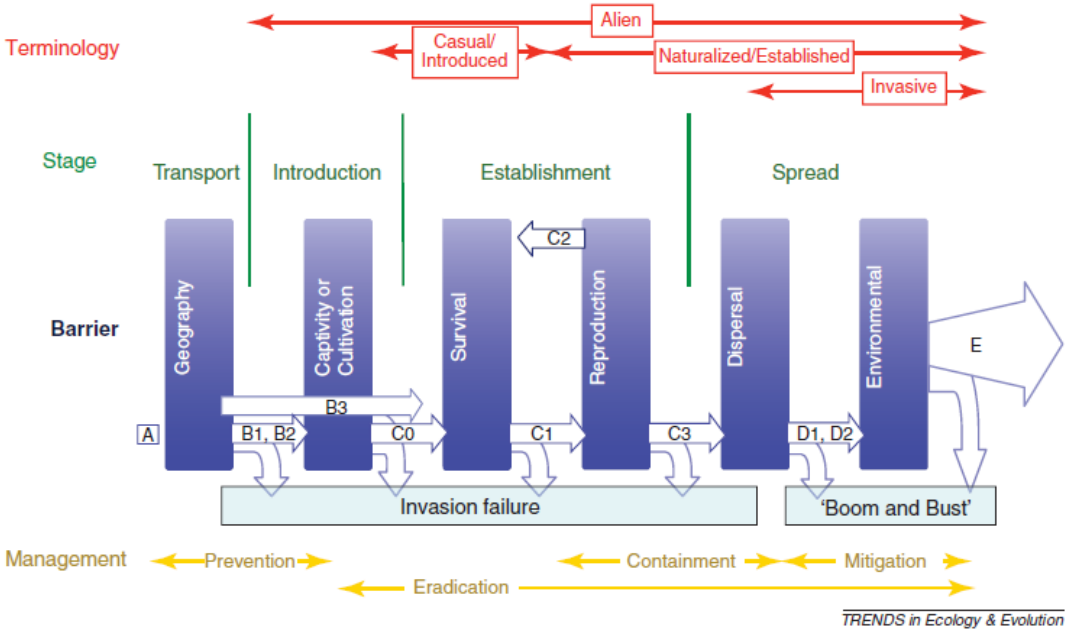


Figure 1.1 - The invasion process can be divided into a series of stages, each representing a barrier to the establishment and spread of the species (or population) in the new habitat. Modified from (Blackburn et al. 2011).

The probability of successful invasion is strongly context-dependent, but alien species traits may represent an important determining factor (Pyšek et al. 2020a). Invasiveness traits may vary across taxonomic groups. For instance, fast life-history strategies characterize successful alien mammals (Capellini et al. 2015), reptiles, amphibians (Allen et al. 2017), but also plants, which grow

faster, produce more seeds, and capitalize more on additional nutrients than non-invasive species (Dawson et al. 2009, 2012). Conversely, alien birds adopt slow reproductive strategies (Sol et al. 2012). Among insects, invasion success is related to a specialist life-style, while in alien birds and mammals, generalist species with adaptable behavior have a higher probability of successful establishment (Sol et al. 2012; González-Suárez et al. 2015).

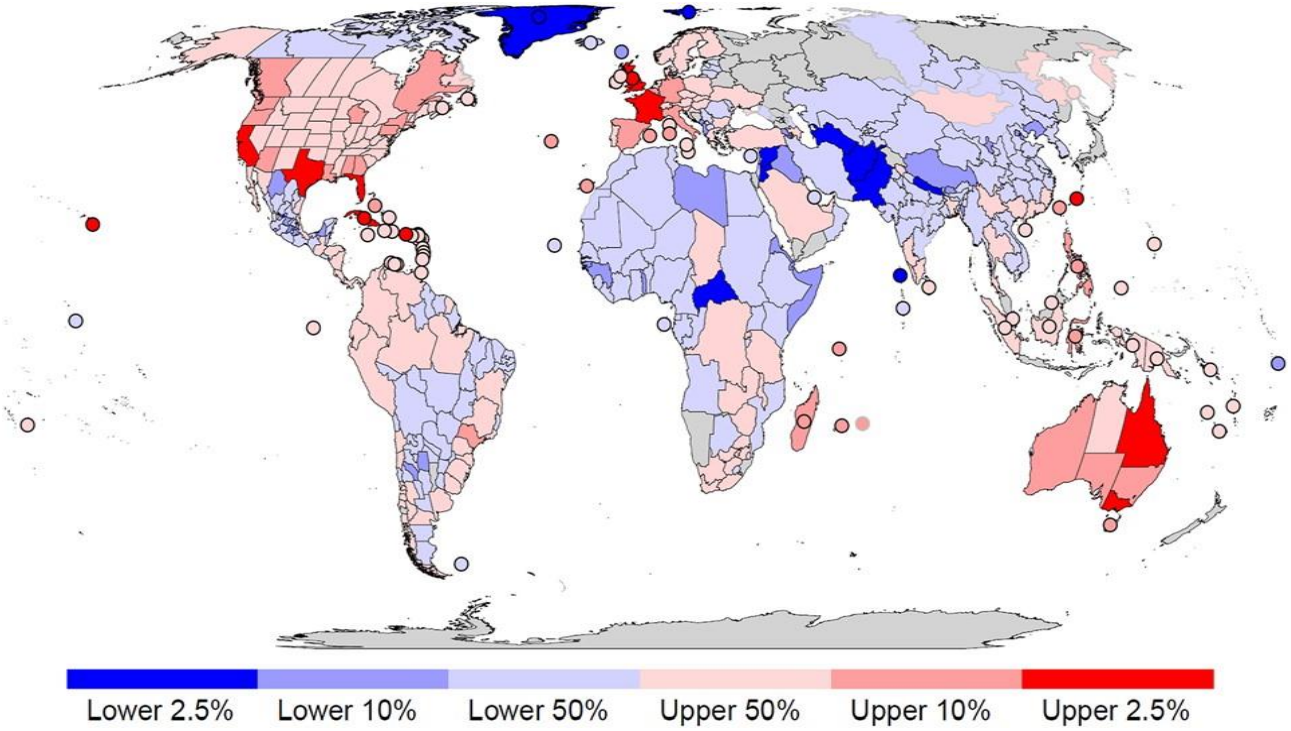
Invasive alien species (IAS) have wide-ranging impacts on both native ecosystems and human societies (Ricciardi et al. 2013; Simberloff et al. 2013; Bellard et al. 2016; Bacher et al. 2018). Impacts may vary consistently in magnitude, and include both positive and negative effects (Jeschke et al. 2014), strongly depending on the ecology of the invasive species and the characteristics of the recipient environments. Negative impacts on ecosystems include competition (e.g., Brown et al. 2002), predation (e.g., Doherty et al. 2016), hybridisation (e.g., Ryan et al. 2009), transmission of diseases to native species (e.g., Miaud et al. 2016), parasitism (e.g., Meeus et al. 2011), poisoning or toxicity (e.g., Shine 2010), biofouling (Shevalkar et al. 2020) or other direct physical disturbance. IAS impacts may also trigger indirect effects on ecosystems (Rodriguez 2006). For instance, in North America, emerald ash borer (*Agrilus planipennis*) induces ash mortality, leading to gap formations and accumulation of woody debris in the forest which determine changes in successional trajectories, facilitate the growth of alien invasive plants, alter ground-dwelling invertebrates communities, and affect bird abundances and foraging behavior (Klooster et al. 2018).

Certain taxonomic groups can exert extremely important impacts on ecosystems and societies; however, impact magnitude largely depends on the characteristics of the recipient environments. As an example, island ecosystems suffered many extinction events due to the predation impact of invasive mammalian predators (Doherty et al. 2016). Island ecosystems, and especially oceanic islands, are particularly vulnerable to IAS impacts (Elton 1958), as they are often characterized by the presence of low-competitive, habitat specialists and/or species lacking efficient antipredatory responses (e.g., Blackburn et al. 2004). Also freshwater ecosystems are greatly affected by invasive species, due to greater habitat connectivity and stronger trophic networks (Moorhouse and Macdonald 2015; Gallardo et al. 2016). For instance, the predation impact of the alien Nile perch (*Lates niloticus*) is responsible for the extinction of at least 200 haplochromines cyclids in the Lake Victoria, where it also changed the habitat, trophic dynamics, and water clarity (Aloo et al. 2017).

On top of the considerable ecological impacts, IAS take a heavy toll also on societies. IAS may threaten human health (Mazza and Tricarico 2018), as is the case of the Asian tiger mosquito (*Aedes albopictus*) which is an important vector of the West Nile Virus and Dengue fever (Benedict et al. 2007), and cause substantial economic losses (Diagne 2020). For instance, the annual cost of invasive invertebrates impacts (principally insects) is estimated at US$8.7 billion (Diagne et al. 2021). However, IAS may also affect material and immaterial assets (Bacher et al. 2018). As an example, the invasive water hyacinth covered vast areas of Lake Victoria, making fishing grounds inaccessible

and forcing people to abandon fishing and emigrate to new areas (Mujingni Epse Cho 2012). In Australia, invasive cane toads caused the local extinction of totemic species used by Aboriginies, leading to the disappearance of sacred celebrations and rituals (Bacher et al. 2018).

Biological invasions have been documented throughout the world, including Antarctica (Hughes et al. 2015). Australia, North America, the Caribbean, and Europe have the highest concentration of reports of established alien species (Fig. 1.2; Pyšek et al. 2020), although the relative threat posed by IAS may vary substantially from one country to another, with highly biodiverse, developing or underdeveloped countries being potentially more threatened, due also to their limited capacity to counter invasive species (Early et al. 2016).



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Despite the problem of biological invasions being recognized since the nineteenth century (e.g., Darwin 1845), the discipline named “ecology of invasions” was officially introduced only in 1958 (Elton 1958), and only in the late 1970s, IAS have been recognized as one of the main drivers of biodiversity loss. This occurred in parallel to the launch of the concept of conservation biology, which took place during a conference organized by biologists Bruce Wilcox and Michael E. Soulé and held at the University of California in 1978. This event sparked the scientific interest in the topic and boosted the launch of initiatives aimed at tackling fundamental gaps of knowledge in the study of biological invasions. Among the most important initiatives is the worldwide assessment of the ecology of biological invasions, launched in 1982 by the Scientific Committee on Problems of the Environment (SCOPE) of the International Council of Scientific Unions (Huenneke et al. 1988), and

the Invasive Species Specialist Group (ISSG) of the IUCN's Commission on Species Survival (SSC) launched in 1993: a global network of scientists and experts whose aim is to 'promote and facilitate the exchange of IAS information and knowledge across the globe and ensures the linkage between knowledge, practice, and policy so that decision making is informed' (Pagad et al. 2015). Another key event that determined the establishment of invasion biology as an emerging field of research was the international conference patronized by the United Nations and the Government of Norway held in Trondheim (Norway) in 1996. This conference led to the establishment of an initiative that was more inter- and trans-disciplinary that the SCOPE programme: the Global Invasive Species Programme (GISP), officially launched in 1997. Following the increased scientific awareness of biological invasions, several international strategies were implemented, such as the Convention on Biological Diversity (held in 1993) and the Global Strategy on Invasive Species (McNeely 2001). It was during the Convention on Biological Diversity that member states were urged to prevent new introductions, and control or eradicate alien species which threaten their native biodiversity.

Over the past two decades, the study of biological invasions has become an increasingly important field of research embracing a growing number of disciplines, ranging from biological to economic and social sciences. Behind this complex and multifaceted field of investigation are the intrinsic context-dependency and temporary nature of biological invasions, which make them (mostly) unpredictable and largely dictated by invasion dynamics (Kumschick et al. 2015).

1.2 Invasion dynamics

Invasion dynamics are defined as 'the action of invasive species and the reaction of recipient socio-ecological systems' (Hui and Richardson 2017). From the side of the invasive species, the spreading dynamics represent a prominent feature of their 'action' on recipient environments. Prior to spreading, biological invasions often face a lag phase (i.e., a dormant state in which the normal spread of the species is not occurring; Crooks 2005), which normally ends when a marked increase in the range size (or records) is observed (Pyšek and Prach 1996). Lag phases may be a direct consequence of the Allee effect (i.e., the reduction in the growth rate of a population at low densities; Stephens et al. 1999), which is caused by inbreeding, demographic stochasticity (e.g., unbalanced sex-ratio), or reduced cooperative interactions (Courchamp et al. 1999), but can also be related to suboptimal habitat conditions and low genetic diversity, subjecting the species to strong directional selection and rapid adaptation to overcome the risk of extinction.

The spreading dynamics of invasive species often reflect their population dynamics, therefore are mediated by population demography, fecundity, mortality, and dispersal capacity. Four main spreading patterns have been identified for IAS: (i) exponential and sigmoidal, (ii) biphasic (iii) linear, and (iv) accelerating range expansion (Fig. 1.3; Hui & Richardson 2017).

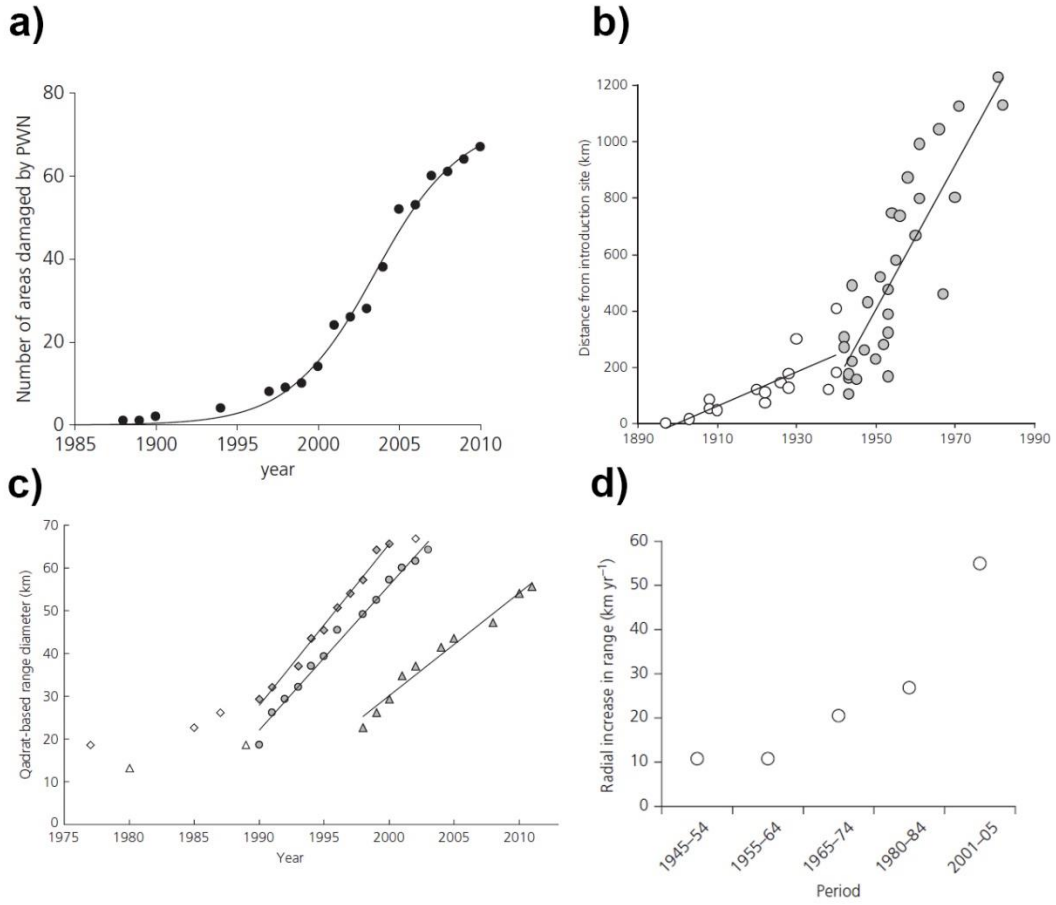

Figure 1.3 - Examples of spreading patterns. (a) Exponential and sigmoidal spreading, showing the number of cities experiencing the disease caused by the pine wilt nematode (PWN, *Bursaphelenchus xylophilus*) in the Korean Peninsula between 1985 and 2010 (Choi and Park 2012); (b) biphasic spreading, showing the range expansion of the Common Starling (*Sturnus vulgaris*) in South Africa during the twentieth century (Hui et al. 2012); (c) linear spreading, showing the rate of spread of eurasian beavers (*Castor fiber*) in the Czech Republic (Barták et al. 2013); (d) accelerating range expansion, where the rate at which the invasion of cane toads (*Rhinella marina*) progressed through tropical Australia (Phillips et al. 2006). Modified from Hui & Richardson (2017).

The exponential/sigmoidal pattern is one of the most commonly observed in invasive species having good dispersal capacities. The exponential phase is the spatial manifestation of a typical Malthusian population model, in which higher core densities are alleviated by the range expansion, while the sigmoidal phase indicates a spread reduced by the limited availability of optimal habitats (e.g., Wehtje 2003) (Fig. 1.3a). A biphasic range expansion (Fig. 1.3b) may sometimes be confused with the previous one. However, in this case, the transition to new habitats and/or the stratified dispersal of the species determine an abrupt shift of spread rate (Shigesada et al. 1995; Liu 2021). Stratified dispersal occurs when long-distance dispersers create satellite populations that later coalesce with the expanding primary population, hastening the process of invasion (e.g., Hui et al. 2012). This phenomenon may also be the result of additional human-mediated introductions (e.g., Siegert et al. 2014). The linear range expansion (Fig. 1.3c) was initially regarded as the null model of invasion dynamics (Skellam 1951), although it is a spreading pattern constrained by limited

dispersal (Hui and Richardson 2017), which is only rarely documented and over limited time periods (e.g., Salo 2005). Lastly, an accelerating range expansion (Fig. 1.3d) can be the result of fat-tailed dispersal of individuals (Kot et al. 1996), which can be observed when the dispersal curve (i.e., the frequency distribution of distances covered by the individuals of a population; Nathan et al. 2003) decreases slower than an exponential curve, indicating a higher frequency of long-distance dispersal movements in the studied population. Alternatively, accelerating range expansion can be due to spatial sorting (Hui and Richardson 2017). This is a spatial evolutionary process driven by intrinsic interindividual differences in dispersal capability, which inevitably results in the accumulation of dispersive phenotypes at the leading edges of an expanding population (Chuang and Peterson 2016). The dispersive phenotypes are then positively selected through assortative mating, leading to a continually increasing expansion rate (Phillips et al. 2010). This process is not regularly observed in expanding populations, as it may be hindered by a demographic Allee effect at the invasion front (Travis and Dytham 2002). Spatial sorting can positively select a broad range of traits, including life history, behavioral, physiological, and morphological traits, but new selective pressure associated with phenotypic trait changes often leads to life-history trade-offs (Chuang and Peterson 2016). For instance, bush crickets (*Conocephalus discolor*) at the invasion front have increased flight ability and endurance, which, however, preclude high reproductive output (Simmons and Thomas 2004).

In addition to population dynamics and dispersal, local abiotic and biotic conditions may strongly determine the spreading patterns (Hui and Richardson 2017). This leads to spatial variation in spreading rates, with the same species exhibiting surprisingly different rates of spread in different parts of the invasive range (e.g., Urban et al. 2008; Lyons and Scheibling 2009).

Although meaningful generalizations on invasion dynamics can be drawn by analyzing different evidence, such as introduction pathways, alien species traits, and the characteristics of the recipient ecosystems (Kueffer et al. 2013; Novoa et al. 2020; Lovell et al. 2021), the high context-specificity of each biological invasion renders invasion dynamics difficult to predict with precision, especially for those groups of taxa which are less studied. Typically, most research efforts have been directed towards the understanding of terrestrial invasions, with most studies focusing on mammals, plants, and insects (Kraus 2009). Conversely, comparatively few studies exist on the invasion dynamics of other taxonomic groups with few recognized extralimital, invasive populations, such as reptiles and amphibians (Capinha et al. 2017).

1.3 Amphibian invasions

Amphibians are one of the less represented taxonomic groups among the invasive alien species (Kraus 2009; Capinha et al. 2017). Established populations of at least 104 species have been reported worldwide (Measey et al. 2016), although new extralimital, introduced populations are continuously being documented, following the unabated global trends of invasions (Seebens et al.

2017). There is a strong taxonomic bias among alien amphibians, with nearly 50% of species belonging to only two families of anurans (Hylidae and Ranidae) and one of caudates (Salamandridae) (Tingley et al. 2010).

Most amphibians' introductions are caused by the pet trade, accidental introduction via cargo transports, or the intentional introduction of species to serve as a source of food or as control agents against pests (Kraus 2009; Measey et al. 2017). Species introduced intentionally are more likely to establish viable populations, but the climatic similarity between native and introduced range is equally important in predicting the establishment success of introduced amphibian species (Rago et al. 2012). Spreading rates of amphibians can be highly variable, depending on species dispersal capacities and characteristics of the recipient environment, with topographic heterogeneity generally having a negative effect (Liu et al. 2014). However, amphibians have been found to spread faster in regions hosting native congeneric species and when they are assisted by human-mediated dispersal (Liu et al. 2014).

Amphibian invasions can have considerable impacts on recipient environments, akin to that reported for invasive fishes and birds (Measey et al. 2016), including toxic effects on native predators, competition, predation, and disease transmission (Kraus 2015; Kumschick et al. 2017). Impacts can also be perceived by human societies, with some species posing a threat to public health and affecting human social life (Measey et al. 2016; Bacher et al. 2018).

The study of amphibian invasions has received increasing attention in recent years, however, much of the research effort on amphibian invasions has been catalyzed by the study on three invasive species: *Rhinella marina*, *Lithobates catesbeianus*, and *Xenopus laevis* (van Wilgen et al. 2018).

Recently, the invasive Asian common toad *Duttaphrynus melanostictus* has received increasing attention (e.g., Measey et al. 2016; Tingley et al. 2018; Bacher et al. 2018), due to the multiple regions it successfully invaded worldwide, but also because it constitutes a relevant biosecurity concern both for invaded areas and for all territories predicted to be suitable for its establishment and spread (Tingley et al. 2018; Andersen et al. 2021). In fact, due to their toxicity bufonids are predicted to have high impacts on recipient environments (Kraus 2015). This emerging invasive species has been documented in the eastern coast of Madagascar (Kolby et al. 2014), a global hotspot of biodiversity, raising concerns for the potential threat that this species can pose to the native populations of potential predators, possibly causing devastating ecological impacts similar (or greater; see Bellard et al. 2014) to those produced by the invasive Cane toad (*Rhinella marina*) in Australia (Shine 2010).

1.4 Madagascar

Madagascar, located in the Indian Ocean, is the fourth largest island in the world (ca 587,000 km^2), and it is separated from Africa by the Mozambique Channel (Goodman and Benstead 2003). Madagascar is a continental island that isolated first from the African mainland (183–158 Mya), then Antarctica (ca. 130 Mya), and finally India, in the late Cretaceous (ca. 68 Mya) (Ali and Aitchison 2008; Vences et al. 2009). Its present-day complex topography underlies a unique bioclimatic zonation. Madagascar is therefore characterized by a mid-altitude mountain ridge running from north to south and creating a steep slope on the east and a gentler slope running from the central highlands towards the west. This main mountain ridge breaks the shallow trade winds and monsoon blowing from the Indian Ocean, ensuring year-round rain to the east, and determining its typical humid tropical conditions, which support the presence of broadleaf evergreen rainforests. Sub-humid climatic conditions in the central highlands determine the presence of sub-humid forests, while, dry deciduous forests and sub-arid spiny forests can be found further west and south, respectively, where increasingly dry conditions are found (Goodman and Benstead 2003). Considering the high-elevation mountains (i.e., above 2,500 m a.s.l.), where ericoid montane thicket is found, Madagascar harbors five main bioclimatic zones. Associated with this bioclimatic diversity is remarkable biodiversity, which makes this island one of the richest hotspots worldwide (Conservation International 2021). Present-day animals and plants of Madagascar originated mostly by dispersal rather than vicariance (see Crottini et al. 2012; Buerki et al. 2013; Welt & Raxworthy 2022), with only a few exceptions (e.g. the Podocnemididae turtles). As such, Malagasy biota is characterized by high rates of endemicity, these endemic groups are generally quite ancient and Madagascar's uniqueness is based on the phylogenetic distinctiveness of higher-level taxa (Yoder and Nowak 2006).

The total number of vascular plant species may be close to 14,000 species, over 90% of which are endemic to Madagascar (Phillipson et al. 2006). Equal levels of endemism can be found among the native faunal groups, with all non-flying mammals and amphibians and 98% of non-marine reptiles being uniquely found in Madagascar (Yoder and Nowak 2006; Glaw and Vences 2007; Crottini et al. 2012).

Among the vertebrates, terrestrial mammals currently count 231 species (IUCN 2021), including the emblematic lemurs (105 species) and tenrecs (31 species). The Malagasy avifauna, with 282 species (IUCN 2021), is considered to be species-poor compared with other continental bird communities inhabiting similar surface areas (Binggeli 2003) but includes interesting examples of adaptive radiation, such as the vangids and the bernierids (Reddy et al. 2012; Younger et al. 2019). Freshwater fishes (excluding introduced species and species from marine and brackish waters), with 97 species (Froese and Pauly 2021), are the least studied vertebrate group of the island (Reinthal and Stiassny 1991) but include the genus *Paretroplus* and the subfamily Ptychochrominae, which are some of the few examples of Malagasy taxa likely originated by vicariance (Irisarri et al. 2018). Reptiles are the richest group on the island (426 species; Uetz et al. 2021), with many

examples of species-rich radiation, such as the endemic pseudoxyrhophiid snakes (89 species; Uetz et al. 2021), or the clade of scincine lizards (65 species; Uetz et al. 2021). However, the most impressive radiation event is found among the amphibians. Mantellid frogs account for more than half of all the amphibian species found on the island (232 out of 369 species; AmphibiaWeb 2021), with almost an equal number of candidate new species that still need to be assessed and formally described (Cocca 2020).

The distribution of biodiversity in Madagascar is strictly associated with the bioclimatic zonation, and, for most taxonomic groups it concentrates in the central and eastern tropical rainforests (Lees and Colwell 2007), while relatively few species can be found in the central highlands (Vences, 2004; Lees and Colwell, 2007; Vences et al., 2009; Brown et al., 2016). However, not all groups respect this pattern of distribution. For instance, important centers of reptile diversity are also found in the western dry deciduous forests and in the southwestern sub-arid spiny forests (Brown et al. 2016).

Multiple species show a restricted distribution range or are locally endemic (Wilmé et al. 2006; Brown et al. 2014), and may be more vulnerable to the risk of extinction. This makes Madagascar one of the most pressing geographic priorities for conservation actions (Myers et al. 2000; Yoder et al. 2005). The main driver of threat for biodiversity is the loss of native forest due to slash-and-burn agriculture, pasture, and the illegal timber trade (Randriamalala and Liu 2010; Scales 2011): fewer than 10% of pristine natural habitats of Madagascar survived since human colonization (Dufils 2003; Harper et al. 2007; Vieilledent et al. 2018). Deforestation also generates forest fragmentation, which further threatens forest communities by changing microclimate conditions, increasing exposure to damaging winds and fires, affecting species composition, and increasing isolation between populations (Vallan 2000; Lehtinen et al. 2003; Riemann et al. 2015). Illegal hunting and invasive species represent other important drivers of threat to the biodiversity of Madagascar, but whereas the former is submitted to national and international wildlife legislation (e.g., CITES), the topic of invasive species has been scantly addressed in Madagascar (Kull et al. 2015).

1.5 Invasive species in Madagascar

The first report on invasive species in Madagascar dates backs to almost a century (Perrier de la Bathie 1928), but the first nearly comprehensive checklist has only been provided at the beginning of this millennium (Binggeli 2003). However, this contribution lacked quantitative data and it only considered the invasive alien plants. Currently, 101 species of invasive plants are reported for Madagascar (Kull et al. 2012), while only partial information is available for animals. Under a conservative estimate, there may be at least 78 species of non-marine invasive animals (Schmidt and Alonso 2005; Dejean et al. 2010; Lopez-Vaamonde et al. 2019; Sileshi et al. 2019, 2022), including domestic exotics (i.e., Malagasy species that established extralimital invasive populations

in other regions of the island; Gehring et al. 2010; Dubos et al. 2014), but only a few studies investigate the impacts and invasion dynamics of these species.

Recently, some attention has been given to the invasive white-footed ant (*Technomyrmex albipes*), and its potential invasiveness of the Malagasy coastal rainforests (Dejean et al. 2010), and, to the invasive marbled crayfish (*Procambarus virginalis*), a parthenogenetic species which has already invaded multiple departments of Madagascar, posing a serious threat to freshwater ecosystems and local livelihoods (Jones et al. 2009; Andriantsoa et al. 2020). This invasive species pushed Malagasy authorities to increase in-country biosecurity measures, which now prohibit the transport of this species across the country (Malagasy Ministry of Agriculture, Livestock and Fisheries 2008). This was one of the first signs of interest in addressing the topic of invasive species by the Malagasy government, which, however, was not backed up by adequate resources and did not bring effective results, yet (Andriantsoa et al. 2019). The absence of a proactive strategy to intercept alien species (e.g., pre-border control measures), allowed several species to enter and establish viable, invasive populations in Madagascar, including the highly problematic Asian common toad. In the face of repeated calls stressing the importance of biosecurity measures to proactively intercept alien species at their entrance into the country (e.g., Randriamoria et al. 2019; Licata et al. 2019, 2020), some progress has been made in this direction (see e.g., VII IUCN World Conservation Congress 2020). Due to globalization, Madagascar is now largely exposed to invasive species, which, besides the negative effects on biodiversity, can constitute a serious threat to both food security (Sileshi et al. 2019) and human health (Mazza and Tricarico 2018).

1.6 Thesis aims and structure

Invasion dynamics and impacts represent the minimum suite of information required to trace a biological invasion, as they provide the basis for quantifying several derived variables and indicators of invasion trends, allowing comparison with other known IAS and the implementation of adequate management measures (Latombe et al. 2017).

The primary aim of this study is to understand these two fundamental aspects of the Asian common toad invasion in Madagascar and provide useful information for its management. To do so, I aimed at responding to the following questions:

1 – What is the extent of the invaded range of the species and the spreading rate of the invasion?
2 – What factors regulate the toads' abundance?
3 – What extrinsic and intrinsic factors drive the spread of this species?
4 – Does spatial sorting of morphological or behavior dispersal-relevant traits occur in this species, and what morphological aspects can provide insights into the invasion dynamics?

5 – Is toad poisoning of native predators already taking place in Madagascar and to what extent? What native predators are more susceptible to toad poisoning?

6 – What is the relationship between the Asian common toad and local human populations, and is there any impact on human well-being and livelihoods?

The thesis is organized into eight chapters. Chapter 2 is complementary to the introduction and will help the reader to achieve an updated image of the toad invasion in Madagascar providing the background knowledge and the problematization of the study system. Here, I provide (i) information on the biology of the Asian common toad, (ii) discuss the potential impacts of toad invasions, (iii) analyze the critical aspects of the management of the toad invasion, and (iv) provide an overview of the available information about the invasion, describing the risk-assessment framework.

Chapter 3 reports a study on the distribution, abundance, and spread of the invasive toad in Madagascar, and provides an insight into the main environmental factors explaining toad abundance. Here, I document the expansion of the invasive range of this species, calculate the invasion spread rate based on distributional records, and provide estimates of toad abundances and habitat preferences. Chapter 4 investigates the morphology of the species to (i) test the hypothesis of spatial sorting of dispersal-relevant morphological traits across the invaded range and (ii) provide insights into the invasion dynamics of this species. Here, I report the factors determining the morphological variation in the invasive population along the invasion gradient. Chapter 5 presents a study on the spatial ecology of this species. Here, I test the hypothesis of spatial sorting of dispersal-relevant behavioral traits, document the movement capabilities, the intrinsic and extrinsic factors determining the spatial behavior, and the habitat preferences of the species. Chapter 6 focuses on the ecological impacts of the Asian common toad, and, in particular, on the toad poisoning effect on a native predatory snake. Here, I estimate the rate of mortality of the Malagasy cat-eyed snake *Madagascarophis colubrinus* caused by toad poisoning and discuss the potential interactions of toads with other native predators, as well as the potential repercussions caused by cat-eyed snakes decline on ecosystems and local populations. Chapter 7 explores the interactions between invasive toads and local communities, highlighting the potential utility of using public surveys' method for rapidly profiling biological invasions in hard-to-monitor regions of the world. Here, I estimate the spreading dynamics and the invasion speed, as well as the environmental factors related to the occurrence of toads, and the potential socioeconomic impacts on local populations. The thesis concludes with a discussion of the obtained results (Chapter 8), drawing conclusions from the multiple approaches used to study the invasion dynamics and impacts of this species in Madagascar. Most of the information generated in this thesis is currently used in the management of the Asian toad invasion in Madagascar and will help characterize the invasion potential of this species both in this biodiversity hotspot and elsewhere.

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Chapter 2

Article 1

The Asian toad (*Duttaphrynus melanostictus*) in Madagascar: A report of an ongoing invasion

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2.1 Abstract

In 2014, an invasive population of the Asian common toad *Duttaphrynus melanostictus* was reported in Toamasina, the major seaport town of Madagascar. This species is toxic, generalist, highly fecund and is rapidly spreading across the lowland habitats of eastern Madagascar. The success of future management strategies will depend on research, public awareness and financial and political support as well as on the feasibility of the methodology to be put in place. Here we present an overview of the Asian toad invasion in Madagascar, tracing the activities undertaken within the framework of the invasion risk assessment in the past 6 years and outlining what needs to be done in the near future to contain the threats to Madagascar's unique biodiversity.

2.2 Introduction

The need to identify biodiversity hotspots emerged as a direct consequence of increasing negative anthropogenic effects on ecosystems, driving scientists and experts to pinpoint priorities for conservation actions (Mittermeier et al. 1998; Myers et al. 2000). The 36 world regions currently identified as biodiversity hotspots represent just 2.4% of Earth's land surface although they support more than half of the world's endemic plant species and nearly 43% of all endemic bird, mammal, reptile and amphibian species (Conservation International 2019). Madagascar was classified as one of the richest and most threatened biodiversity hotspots worldwide, with only about 10% of its

ecosystems persisting in a pristine or semi-pristine state (Myers et al. 2000; Goodman and Benstead 2005). Madagascar's uniqueness is characterized not only by the total number of species but also by the higher level of phylogenetic distinctiveness of its endemic families and genera (Ganzhorn et al. 2014). For example, among the extant vertebrates, the majority of the lineages succeeded in colonizing Madagascar through sea dispersal, during the Cretaceous – Cenozoic times, when ocean currents were particularly favourable for dispersion (Samonds et al. 2013) and when Madagascar experienced major adaptive radiations, which determined the composition of the present-day vertebrate assemblage (Goodman and Benstead 2003; Vences et al. 2003; Crottini et al. 2012; Samonds et al. 2012, 2013; Ganzhorn et al. 2014)

Anthropogenic habitat destruction and fragmentation have been particularly severe during the last decades (Harper et al. 2007; Vieilledent et al. 2018) and continue to be the biggest threat to the survival of Madagascar's biota. Among other threats, the impact of invasive species is emerging as an important factor impacting Madagascar's unique biota (Kull et al. 2015). Biological invasions take place when alien species are introduced to new regions, establish themselves and spread to new environments (Blackburn et al. 2011). This human-mediated phenomenon has severe impacts on human society and is one of the major direct drivers of ecosystem changes, having an ever- increasing impact on biodiversity and ecosystem services (Sarukhan et al. 2005). More than 50 animal and 1200 plant alien species are now documented in Madagascar (e.g., Binggeli, 2003; Ghulam et al., 2014; Goodman et al., 2017; Irwin et al., 2010; Kull et al., 2015, 2012; Rakotomanana et al., 2013), and only a small fraction has been classified as invasive (Kull et al. 2015; Goodman et al. 2017). Complicating the issue further is the severe lack of border biosecurity practices and infrastructure that could help to prevent alien species introduction at the boarder (Reardon et al. 2018).

The Asian common toad (from here onwards referred to as 'Asian toad') *Duttaphrynus melanostictus* (Schneider 1799) (Fig. 2.1) was first discovered by the scientific community in Madagascar in March 2014 (Kolby et al. 2014), but its initial introduction is believed to have begun towards the end of the first decade of the twenty-first century (McClelland et al. 2015; Moore et al. 2015). This first report prompted some researchers to speculate on the possible risks associated with the management of this invasion (Mecke 2014), who were reassured by a following contribution by Andreone (2014). The Asian toad invasion is likely the result of the accidental introduction of a few individuals via commercial shipping containers from Southeast Asia (McClelland et al. 2015; Vences et al. 2017). After a lag phase of a few years, the Asian toad population expanded its range exponentially, and the apparent rate of spread continues unabated (Moore et al. 2015; Licata et al. 2019). Conservation biologists fear that this invasion will parallel the infamous invasion history of the cane toad *Rhinella marina* (Linnaeus 1758) in Australia, which has impacted native species communities (Shine 2010).

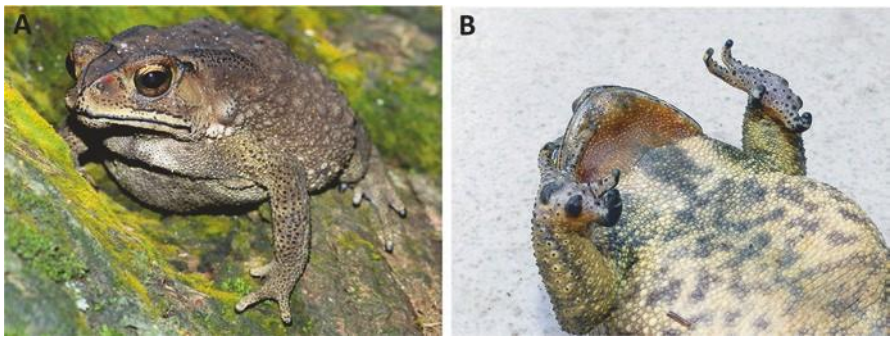

Fig. 2.1 - (a) *Duttaphrynus melanostictus* in Toamasina; (b) male *D. melanostictus* showing secondary characters, namely, the yellow-orange subgular vocal sacs and the nuptial pads on the first and second fingers. (Photo A by F. Andreone; B by A. Crottini)

2.3 Natural History of the Asian Toad

The Asian toad belongs to the large and widely distributed family Bufonidae (Van Bocxlaer et al. 2010), also known as true toads. True toads have a natural worldwide distribution that includes the Americas, Eurasia and Africa, and their evolution has been mostly explained by a gradual adaptation towards a phenotype that is particularity suitable for colonizing new habitats (Duellman and Trueb 1994; Pennisi 2010; Van Bocxlaer et al. 2010).

The Asian toad is terrestrial, has crepuscular/nocturnal habits, is an ecological generalist, possesses parotoid glands that secrete bufadienolide toxin and has a strong invasive potential due to its tolerance for disturbed environments, as already documented in many other regions worldwide, both in its native and invasive range (Csurhes 2016; Reilly et al. 2017). This species is native to Southeast Asia, but after accidental introductions, it had invaded many other areas, which now include Bali, New Guinea, Sulawesi, Timor-Leste, Maldives, several other small islands of the Sundaic region and Madagascar (Church, 1960; Gardiner, 1906; Kolby et al., 2014; Reilly et al., 2017; van Dijk et al., 2004, Fig. 2.2). Recent phylogeographic analyses have revealed this species to be a complex of at least three distinct evolutionary lineages that are genetically and ecologically different and have discrete distributions (Asian mainland, coastal Myanmar and the Sundaic islands; Wogan et al., 2016). The Asian toad is most commonly found in disturbed lowland habitats (Hendrix et al. 2008), although they have been observed in deep forest in Thailand, Borneo and Sri Lanka (JR, pers. obs.). It is commonly found in rural and urban areas, often associated with human habitation (van Dijk et al. 2004), and it can be considered a habitat generalist in terms of breeding requirements (Daniel and Sekar 1989; Saidapur and Girish 2001).

The Asian toad is a typical large and stout toad, with rather short and strong legs and thick, dry skin covered with warts, featured by characteristic black bony ridges on the head. Individuals can grow up to 200 mm snout-vent length, with a mean length of 78 mm in Madagascar (FL, pers. obs.). Females are generally larger than males, as is standard for most anuran species. The colouration of *D. melanostictus* varies from light cream to almost black, with brick-red tinges, but the most common pattern is pale yellow-brown streaked with black or reddish-brown spots. Males

show a yellowish orange subgular vocal sac, and during the breeding season, they also exhibit blackish nuptial pads on the first and second fingers (Fig. 2.1).

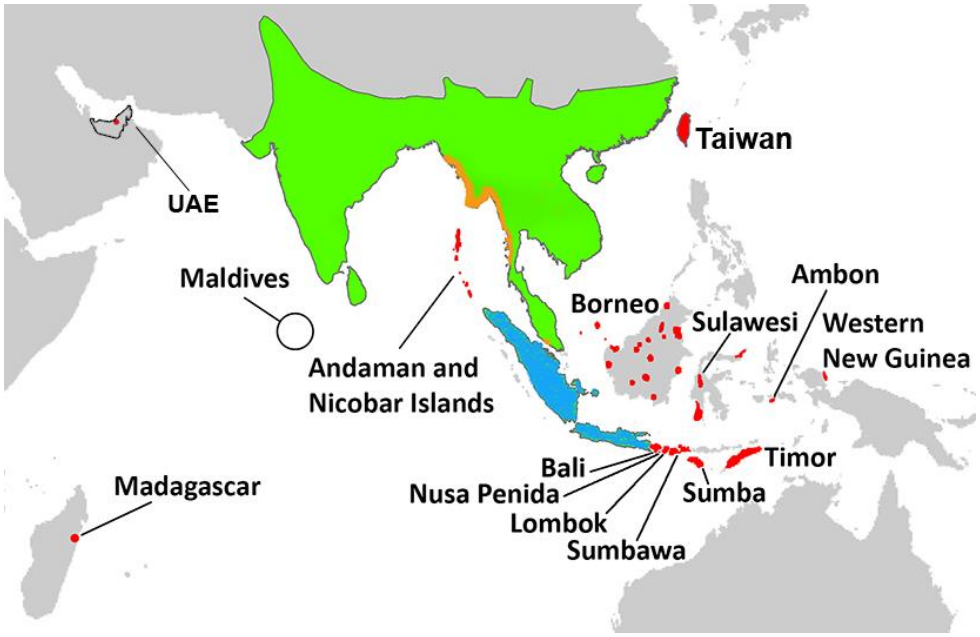

Fig. 2.2 - Map showing the invasive (red) and the native distribution of the three principal clades of the *Duttaphrynus melanostictus* complex. In green, mainland lineage; in orange, coastal lineage; in blue, insular lineage. (Modified from van Dijk et al. 2004 and Wogan et al. 2016)

As for many other bufonid species, females usually reach sexual maturity at a later age than males (Ngo and Ngo 2013). A skeletochronological study on a native population of Asian toad in India reported that this species can live up to 12 years in the wild (Nayak et al. 2007). Interestingly, preliminary skeletochronological analysis of the invasive population in Madagascar failed to identify *lines of arrested growth* (*LAGs)* in either phalanges or femurs, suggesting that in Madagascar they don't undergo seasonal changes in physiological growth (F.M. Guarino, pers. comm. 2015). In Vietnam, Asian toads breed once per year, following heavy rainfall (Ngo and Ngo 2013). In Madagascar it is still not certain if the invasive toad population breeds only once or it continues to breed throughout the year. Breeding and oviposition usually occur in still water, but also in slow-flowing rivers and ephemeral water bodies, where females can lay up to 10,000 eggs (FL, pers. obs.) arranged in long single-row strings. In their native range, tadpoles require 18–20 days to reach metamorphosis (Mahapatra et al. 2017) and they are known to metamorphose faster when they are in kin groups (Saidapur and Girish 2000, 2001). Tadpoles have an opportunistic diet, and cannibalism has been reported on conspecific eggs, tadpoles (of different developmental stages) and adult carrion, as well as on tadpoles of other species (Mahapatra et al. 2017). Adult toads are considered to be generalist feeders, and their diet is mainly composed of terrestrial invertebrates, with a strong prevalence of ants and termites (Berry and Bullock 1962; Mercy 1999; Hui 2015; Döring et al. 2017).

One of the characteristics that make this species a successful invader is its toxicity (Marshall et al. 2018). Most bufonids secrete potent toxins (bufadienolides) to defend themselves from

predators (Chen and Kovaříková 1967; Ujvari et al. 2015). These toxins are secreted by the skin, in particular by the post-orbital parotoid glands (Vitt and Caldwell 2013). The toxin inhibits the sodium-potassium pump of cardiac cells, thus impeding the ion-transport mechanism, causing potentially lethal cardiotoxic effects (Lingrel 2010). Despite numerous instances of resistance to bufadienolides across the animal kingdom, a recent study revealed the likely widespread vulnerability of potential predators to the bufotoxins of the introduced Asian toad in Madagascar (Marshall et al. 2018).

2.4 Ecological impacts of toad invasions

Alien bufonids (i.e., true toads) have the highest impacts on ecosystems and societies (Measey et al. 2016). Most examples of ecological impacts of toad invasions come from the invasion history of cane toads (*Rhinella marina*) in Australia. Imported in 1935 to control sugar cane pests in Queensland, cane toads have spread throughout much of the wet-dry tropical habitats of North Australia, recently crossing the border into Western Australia (Urban et al. 2008). Due to its generalist and opportunistic diet, large size, high fecundity, rapid development and tolerance of broad climatic and environmental conditions, cane toads succeeded in reaching very high densities (Freeland 1986) and have successfully colonized a large proportion of Australia (Urban et al. 2007). The large array of interactions this invasive species has with Australian native ecosystems gave rise to one of the most extensive scientific investigations of a biological invasion (e.g., Doody et al., 2015; Lever, 2001; Phillips et al., 2010; Shine, 2010; Shine and Brown, 2008) and provides us with an insight into the problems that could emerge in Madagascar with the invasion of *D. melanostictus*. The first and most serious negative effect invasive cane toads have on Australian native communities is the lethal poisoning of native mesopredators following the ingestion of the toxic toad (Shine 2010; Ujvari et al. 2015). Australian predators have no evolutionary defense against toad toxins, likely because bufonids have not naturally colonized Australia, and although some species showed some degree of tolerance to bufotoxins (Phillips et al. 2003), the vast majority of Australian native species are susceptible (Shine 2010). Cane toads are toxic across all life stages, and their toxicity is effective both in aquatic and terrestrial environments (Lever 2001; Shine 2010). In the aquatic environment, mass mortality events or declines have been documented among freshwater fishes (Grace and Sawyer 2008), tadpoles of native frog species (Crossland et al. 2008; Crossland and Shine 2010), and aquatic invertebrates such as snails and leeches (Crossland and Alford 1998). These lethal effects are repeated in terrestrial environments, where dramatic declines of predator communities have been observed, especially among crocodiles, lizards, snakes, birds and mammals (Lever 2001; Shine 2010). Other documented negative effects of this invasion include the alteration of native invertebrate communities. Due to high cane toad densities, they have a strong impact on the abundance and species richness of native arthropods, and because they are

almost 'invulnerable' to predators in their invasive ranges, they reduce the rates of nutrient recycling in ecosystems, by acting as a major nutrient sink (Greenlees et al. 2006). Cane toads are also known to directly interfere with the behaviour of native species. For instance, Australian native frogs tend to avoid refuge sites previously used by cane toads because of the chemical cues produced by their skin that persist in the ecosystem (Pizzatto and Shine 2009). Other negative effects are related to both cane toads' high fecundity and abundance (Shine 2010). In particular, high densities of cane toad tadpoles, especially in temporary water bodies, can promote the expression of the cannibalistic phenotype (Polis 1981; Crump 1983), increase competition for natural resources and affect the growth of native tadpoles that occur syntopically (Williamson 1999; Crossland et al. 2009).

Last but not least, alien toads can bring pathogens and parasites from their native ranges and transfer them to the invaded ecosystems (Delvinquier and Freeland 1988; Dubey and Shine 2008), potentially causing epidemic infections among native amphibians (e.g., Daszak et al., 2004; Garner et al., 2006; Yap et al., 2018).

2.5 A critical analysis of the management of the toad invasion in Madagascar: What went wrong?

The unexpected discovery in March 2014 of a naturalized population of the Asian toad, locally called Radaka boka, around Toamasina (on the east coast of Madagascar) created great concern among the scientific community. There was a call for rapid efforts to eradicate the Asian toad (Kolby et al. 2014; Andreone 2014) and a rapid mobilization of the conservation community working with the amphibians of Madagascar to identify resources to develop a strategic plan for the eradication of the invasive species (Crottini et al. 2014a). The concerted effort of amphibian and invasive species biologists led to the realization of a feasibility plan for eradication, released the following year (McClelland et al. 2015), which was followed by field tests of potential eradication methods and to provide further information for evaluating eradication feasibility (Reardon et al. 2018). In the meantime, the multiple calls for action coming from the scientific community (e.g., Kolby et al., 2014; Kull et al., 2015; Pearson, 2015) and the media led some authors to publish a note which 'caution[ed] against disproportionate countermeasures' (Mecke 2014). This was immediately followed by Andreone et al. (2014), advocating for the implementation of swift, although carefully weighed actions (a standard procedure for invasive species management experts) to tackle this toad invasion, as a rapid response is often the only opportunity to obtain effective results (Kraus 2009; Clout and Williams 2009). A key point raised by Reardon et al. (2018) was that so long as the invasive species remained contained within habitat with low ecological value and no rare or locally endemic species, any nontarget impacts of eradication tools may be ethically justified. However, with ongoing delays in making operational decisions, the risk that the toad would spread to occupy

more ecologically intact habitats where eradication tools would become limited due to unacceptable nontarget risks would increase and therefore seriously diminish any probability of eradication success.

After the first hectic months of planning and fundraising, in June 2014 official working groups were set up in an effort to streamline communications and decision-making. The groups covered topics such as communication, fundraising, distribution surveys, education, the eradication feasibility study, disease screening and genetic studies. These working groups later became the backbone of a national committee tasked with managing the response for the invasive toad phenomena. The committee consisted of representatives of major stakeholders from the Malagasy government, private enterprises and the conservation NGO sector and from August 2015 was coordinated by Madagascar Fauna and Flora Group (MFG).

Visual-encounter surveys to identify the extent of occurrence of the invasive toad started in May 2014, as part of the collection of preliminary information needed for development of the eradication feasibility study (Crottini et al. 2014a; McClelland et al. 2015; Moore et al. 2015). These delimitation surveys showed that the Asian toad already occupied an area of at least 108 km^2 in the south and southwest areas of Toamasina (Moore et al. 2015). The centre of distribution of the toad—and the earliest reports of the toads' occurrence—was identified in very close proximity to a nickel and cobalt refinery based in the south of Toamasina. Based on the reports of local residents, it was suggested that the toads arrived with construction equipment sometime between 2007 and 2011, when an enormous amount of material was imported from Southeast Asia to this rural site. The consistency of these observations, along with the outcomes of the field-based research, helped to narrow down the probable point of introduction of the species (Moore et al. 2015).

With the release of the eradication feasibility study (McClelland et al. 2015), it became clear that the alien toad population in Madagascar was already extremely large, with a density of up to 51 toads per 100 m^2 reported for the urban area and around 580/ha across all sites surveyed (McClelland et al. 2015). The toad was reported in four different ecotypes: urban, rural/agricultural, palm-oil plantation and non-production forest habitat, locally called savoka (consisting of degraded forest and mixed scrubland, McClelland et al., 2015; Moore et al., 2015; see Fig. 2.3). These preliminary results led to the conclusion that an eradication would likely no longer be feasible, due to the lack of easily implemented, effective and scalable methods proven to efficiently eradicate large amphibian populations, and the lack of available techniques to reliably detect individual toads at low densities and the infeasibility of introducing effective biosecurity measures within Madagascar to prevent transport of the toad to areas outside the present invaded area (McClelland et al. 2015). In light of these considerations, it was suggested that the window of opportunity for succeeding in a full eradication was closing fast (McClelland et al. 2015). This was later confirmed by Reardon et al. (2018), who also highlighted social, political and logistical challenges (including lack of sufficient funds) as reasons to regard the eradication of Asian toads from Madagascar as infeasible.

Reardon et al. (2018) also identified the urgent need for improved border biosecurity policy and infrastructure to prevent further incursions of this and other invasive species. With the eradication considered unfeasible, the national committee decided that the next steps should be to look at identifying site-specific programs, focusing on mitigation efforts to protect key areas of particular conservation concern and native species at risk, using these efforts to gain more experience on potential management options for this invasive species, integrating the tools tested by Reardon et al. (2018). Initiatives were directed to also promote the development of border and internal biosecurity programs aiming to prevent new invasions or reinvasions.

Fig. 2.3 - Range of habitats occupied by the Asian toad in Toamasina. (**a**) urban; (**b**) degraded urban habitat with garbage pile on outskirts of town; (**c**) rural area mixed with degraded forest and mixed shrubland, or 'savoka'; (**d**) oil-palm plantation. (Photos by F. Licata)

There are valuable lessons to be learned from the difficulties encountered during the initial years of the Asian toad invasion response that should be heeded to allow a faster and more effective response in the event of a new invasive species being introduced to Madagascar. For example, the national committee faced a number of challenges that ultimately led to its efficacy being greatly hampered: despite initial good intentions to be inclusive, the committee's size meant that it was difficult to reach consensual decisions, and the national committee was never officially ratified by the Malagasy government, so there was no official authority to execute and enforce agreed-upon urgently needed plans; all people working on the toad issue were doing so in their spare time in addition to their full-time jobs, which led to a frustratingly slow pace of progress, in 2014, and at the time of the first report of the toad in Madagascar, there was no designated government agency specifically responsible for dealing with invasive-species issues, and it remains unclear whether the toad issue falls under the jurisdiction of the regional or national government authorities. However, most of the encountered limitations are common to the ones encountered during other attempted

herpetological eradications elsewhere (Kraus 2009). In the specific case of Madagascar, the limited governmental function and the partial administrative decentralization of political decision-making powers has resulted in a lack of clear jurisdictional authority between the regional and national government bodies in some instances. This exacerbates the debate between the different (local and central) institutions, which sometimes results in delays in the execution of local policies (Tahina 2015; World Bank 2018), such as has been the case with the current toad invasion response. Also, there remains no clear mechanism whereby international help and support from entities such as the IUCN can easily be accessed to effectively tackle urgent invasive-species issues and which are the possibilities for the organizations operating in the invaded areas.

Although we are aware of the difficulties in developing something of this kind, we believe that the creation of international rapid-response capacity for invasive species could be extremely useful, at least to provide advice and initial support to countries lacking their own protocols or biosecurity networks. There are a number of international list servers that function in this way but all rely on the initiative of in-country actors. To enable timely responses within a timeframe that allows eradication to be a viable option, it is necessary to have a designated and dedicated ministerial team whose sole responsibility is to deal with broader biosecurity and invasive-species issues. To this end, the authors welcome the recent appointment of a professional figure within the Ministry of Environment and Sustainable Development (*Ministère de l'Environnement et du Développement Durable,* MEDD) to be responsible for invasive-species issues. Capacity-building for invasive species management and biosecurity is required to support this post and to develop a network of specialist experts within Madagascar itself to deal with arising threats from invasive species and to work towards strengthened border biosecurity and supporting policy.

2.6 The risk-assessment framework and a plea for applied research

2.6.1 Latest news from the invasion front

Faced with a very difficult (if not impossible) eradication, it became imperative to identify and evaluate suitable eradication tools to finalize the assessment of feasibility and to provide data from which mitigation efforts could be informed. Many different methods have been tested on cane-toad populations in Australia, among which the use of parotoid-gland secretions to attract tadpoles (Crossland and Shine 2011) and acoustic lures for adult toads (Schwarzkopf and Alford 2007), stand out as potentially effective methods to employ, especially in contrast to the efficacy of traditional control tools, such as drift fences and pitfall traps. In early 2016, a series of techniques was tested on Asian toads in Madagascar to assess the efficacy of potential eradication tools (Reardon et al. 2018). Tested methods included hand capture, pitfall traps and drift fences, the use of baited tadpole traps and citric- acid spraying (Reardon et al. 2018). The results of this comparative

study showed that chemical luring of tadpoles was ineffective, but Reardon et al. (2018) recommended further tests at different stages of larval development. The study showed that citric-acid spraying was surprisingly effective in killing adult and subadult toads, similar to its successful use for controlling other invasive anurans (Beachy et al. 2011). However, citric-acid spray, as well as being socially and logistically complex to employ, was regarded as a viable choice only in areas of extreme ecological degradation due to nontarget impacts on other fauna (Reardon et al. 2018). Drift fencing and pit-fall trapping proved to be moderately effective and simple to employ and also providing the chance to collect important demographic information (Reardon et al. 2018). These data were used to infer toad densities, which were found to be the highest (1266 toads/ha) in degraded urban habitats, whereas in forested habitats, the number estimated on the basis of counted individuals dropped down to zero (Reardon et al. 2018). Total abundance in 2016 was estimated to be 7.2 million toads, in comparison to the 3.77 million toads estimated with data collected in 2014 (see McClelland et al., 2015; Reardon et al., 2018). Sadly, this number is expected to increase more quickly each year, confirming again the tremendous efforts and rapid intervention needed for eradication. Hand capture was also tested within non-contained, replicated 9 ha areas. This effort was largely a response to the frequent but doubtful suggestion that a public bounty for toads could facilitate eradication (Goodman et al. 2017). Putting aside the issue of bounty subversion (the possibility that toads would be sustainably harvested or farmed for pay in such an economically poor environment), the method failed to demonstrate a significant decline in capture rates (Reardon et al. 2018).

The most recent field data (collected in September 2017) reports alarming rates of dispersal, with toads found more than 20 km away from the presumed point of introduction (Fig. 2.4b), meaning that the invasion front has advanced around 3.3 km/year, if we assume that the invasion started in 2010 (Licata et al. 2019). This highlights the marked increase of invasion rate for this species in comparison to the first surveys from 2014 (Moore et al. 2015), for which the dispersal rate was inferred to be 2 km/year (McClelland et al. 2015). However, this value is still consistently lower than the spreading rates of cane toads in Australia, where the invasion front advances up to 60 km per year (Phillips et al. 2007; Urban et al. 2008; Estoup et al. 2010; Brown et al. 2011).

Toad populations are currently expanding in all landward directions probably facilitated by the widespread presence of human settlements in the area, with more than 2000 villages or small groups of huts counted in the invaded area (FL, pers. obs.), which are known to be favourable habitats for the species (Licata et al. 2019). The southward invasion appears to have been facilitated by the presence of multiple man-made canals and waterways of lentic character (Rahel 2007), which allowed the Asian toad to cross the Ivondro River by 2014 (Moore et al. 2015). Conversely, it seems that the northward invasion has been slowed down by the Ivoloina River, raising the possibility that this river might function as an ecological barrier, as already reported in the literature for other river systems (Leblois et al. 2000; Li et al. 2009). However, considering the relatively slow flow of this river,

it seems that if it is acting as a barrier, it is likely to hold back the dispersal of toads for only a brief time. The river is crossed by several bridges and the quantity of freight travelling north from the invaded area guarantees the spread of the toads north even if the river acts as a temporary barrier to dispersal. The toad's invasion also seems to be advancing rapidly towards the interior of Madagascar, getting closer to some of the best- known biodiversity strongholds of the eastern coast of Madagascar, such as the Betampona Strict Nature Reserve (now less than 30 km northwest of the invasion front).

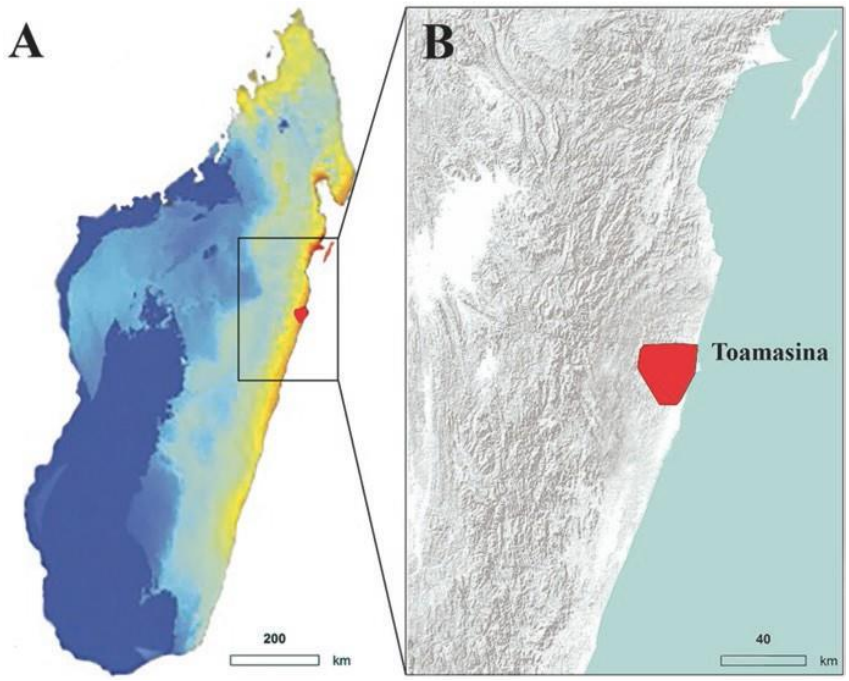

Fig. 2.4 - (a) Species-distribution model based on occurrence data of the distribution range of the Southeast Asian lineage of the *Duttaphrynus melanostictus* complex. Warmer colours indicate climatically suitable areas. (Modified from Vences et al. 2017); (b) Close-up of the eastern coast of Madagascar showing the currently estimated invaded range of the Asian toad (based on occurrence data collected up to late September 2017). (Modified from Licata et al. 2019)

Between the end of 2016 and the beginning of 2017, *N*-mixture models (Royle 2004; Ficetola et al. 2018) were used to estimate more accurately the size of the Asian toad population (Licata et al. 2019). Abundances were computed at six sites across the invaded area and results showed strong variation across the surveyed sites, reflecting the findings of Reardon et al. (2018) with an average of 184 toads per hectare (Licata et al. 2019) and maximum abundance values recorded in a palm oil plantation (559 toads/ha, Licata et al., 2019). These correlative models were also used to determine the influence on toad abundances of some of the potentially most important environmental factors observed on the ground (Licata et al. 2019). This study suggests that rubbish dumps and organic waste presence have a strong positive effect on toad abundances, probably because they provide safe shelters, high numbers of potential prey and favourable microclimate conditions. Conversely, the proximity to roads seems to be related to lower toad abundances, as does forest cover, whose influences, however, need to be further investigated (Licata et al. 2019).

Understanding the spatial and demographic parameters will help to strategize the future management of the species, since it will enable the identification of possible impediments to its dispersion and it will help inform containment and delimitation efforts in these areas.

2.6.2 Modelling future scenarios of the invasion

A first attempt to estimate the invasive potential of the Asian toad in Madagascar was based on environmental niche modelling, which used bioclimatic and occurrence data of *D. melanostictus* in its native range, revealing that the climate of Madagascar was particularly suitable for the Asian toad to thrive (see Fig. 2.1 in Pearson, 2015). Subsequently, Wogan et al. (2016) revealed that *D. melanostictus* consists of at least three distinct evolutionary lineages, each having a narrow geographical range and ecological niche. The data that became available from this study helped to study the invasion history of the invasive population of *D. melanostictus* in Madagascar, which was suggested to result from a single source population either from Vietnam or Cambodia (probably the Ho-Chi-Minh-City area; see Fig. 2.1 in Vences et al., 2017). Species distribution models derived from the use of only the bioclimatic and occurrence data of the Southeast Asian lineage, restricted the suitable climate space for the Asian toad in Madagascar to the lowlands of the eastern and north-western coasts of Madagascar, an area which is almost entirely included into the predicted climate space of the Southeast Asian lineage (see Fig. 2.4).

2.6.3 Investigating potential impacts

Ecosystem changes determined by this invasion seem likely to have a strong impact on the native fauna of Madagascar. The poisoning of native predators is certainly one of the most feared consequences of this invasion (Crottini et al. 2014a; Kolby et al. 2014), especially after that Marshall et al. (2018) predicted the vulnerability of most Madagascan predatory birds, mammals, amphibians and reptiles to the toads' toxins (see Fig. 2.1 in Marshall et al., 2018).

At present it is difficult to predict the extent of impacts on native species, depending, as it does, on the natural histories of the native predators and on the toad's ability to adapt to different habitats, such as rainforest (Marshall et al. 2018). Using a multivariate niche modelling approach, Brown et al. (2016) forecast that the niche overlap between the invasive Asian toad and six potential carnivore predators in eastern Madagascar is sufficient that they were at high risk of rapid decline if the Asian toad becomes part of their diet. The authors suggested that a strategic plan to monitor the endemic and most threatened carnivores should be planned to prevent their extinction and proposed that monitoring the brown-tailed mongoose, *Salanoia concolor*, should be a priority due to its limited distribution (Goodman 2012). To strengthen the efficacy of this proposed conservation measure, the development and implementation of a plan for the early detection and rapid response

of recently established populations of toads should also be considered. We think this can be achieved with the establishment of citizen-science campaigns, aimed at informing the community on the importance of the unique biodiversity of Madagascar to maintain the ecosystem services and on the importance of reporting the presence of a dangerous invasive species that can dramatically modify these services, although there are significant barriers to such a strategy due to the limited communications infrastructure and poverty of the rural population.

Further, we believe it is imperative to design containment methods to defend ecologically sensitive areas that are, or will soon be, encroached upon by the invasion front. This may consist of the establishment of fences or barriers against toads, which will, however, need constant monitoring, as a single breach would compromise their functionality (Tingley et al. 2017; Reardon et al. 2018). Other possibilities to evaluate include the use of male advertisement calls to attract females, targeted female removal and traps with acoustic lures during the breeding season (Tingley et al. 2017).

Besides the aforementioned threats, the Asian toads may have also introduced new parasites and pathogens, and it will be important to determine whether, for example, they are carrying *Batrachochytrium dendrobatidis* and *Ranavirus,* among other potential pathogens. Recent results indicate striking differences between the bacterial communities of *Duttaphrynus melanostictus* and a native co-occurring species (Santos et al. 2021), with the toad microbiome being enriched for xenobiotic biodegradation and metabolism processes (Santos et al. 2021). The microbiome on amphibian skin plays an essential role in disease resistance, host health and adaptation to biotic and abiotic stresses (Wang et al. 2017; Jiménez and Sommer 2017; Campbell et al. 2019), and the observed differences could be related to the higher capacity of the toad to cope with environmental alteration and anthropogenic stress (Claus et al. 2016).

Other studies are currently underway: radiotelemetry is currently applied to ascertain whether the toad is likely to use forested habitats and to gather information on its dispersal capabilities and the toads' interactions with native predators. These data, combined with fine-scale habitat mapping through remote sensing techniques (Comiso 2010) and toad presence-absence data, will be used to inform specific individual-based models of dispersal processes and will enable the prediction of likely future spread scenarios and identify the areas of higher risk of invasion.

2.7 Future Steps

The Asian toad is emerging as a very successful invader (Reilly et al. 2017). Being a human commensal, it is often found close to human settlements, further increasing its chances of being accidentally translocated to new areas. Stowing away in freight and shipping containers is one of the most frequent pathways of introduction for many alien species and is likely to become an ever-increasing risk during the forthcoming years (Hulme 2015; Tingley et al. 2018). Asian toads are

frequently reported in shipping containers, raising the fear this species will invade other areas (Measey et al. 2017; Tingley et al. 2018; Mo 2018). This poses a threat both for countries with active biosecurity programmes that are able to modify or strengthen their protection tools to prevent new invasions and much more so for countries lacking biosecurity infrastructure and capacity, such as Madagascar. On the national scale, the seaport city of Toamasina has more than 170,000 containers per year passing through its port facilities (World Bank 2018), and tons of goods are moved daily from Toamasina across the entire island, highlighting fears that this will result in the translocation of this (and likely other) invasive species to other parts of the country or to other countries. Many products (such as lychees and bananas) are regularly exported from Toamasina and could easily reach other jurisdictions. Lychees represent an important fruit export from Madagascar, and many countries (including several EU countries) receive fruits collected and stocked from the Toamasina area (Jahiel et al. 2014). The potential presence of living Asian toads within these will become increasingly likely and should be the focus of internal biosecurity practices. On a more local scale, agricultural practices are thought to play a critical role in promoting Asian toad colonization. For example, in a palm-oil plantation close to Toamasina, where toads reach high densities (see Sect. 2.5.1), tons of plant remains are used as mulch for young palm groves (Hamdan et al. 1998). Asian toads profit from the shelter this substrate provides (FL, pers. obs.), and during the relocation of the mulch across plantations, toads are very likely to be moved into new palm oil parcels.

Non-governmental organizations are important civil-society actors and could represent key players in mitigating biodiversity crises. The local NGO 'Madagascar Fauna and Flora Group' (MFG), with its headquarters in Toamasina, has played a strategic role in responding to this invasion since its first report, setting up the basis for the risk-assessment framework that was described here and carrying out initial distribution surveys in collaboration with another local NGO, Association Mitsinjo. MFG promptly launched a campaign using radio broadcasts, posters (Fig. 2.5) and village visits to raise local awareness about the presence of this noxious species; they also created a toll-free hotline number that local people could use to report the presence of the toad during the initial stages of the delimitation effort for the toad, which is now suspended because it was found to be of limited value.

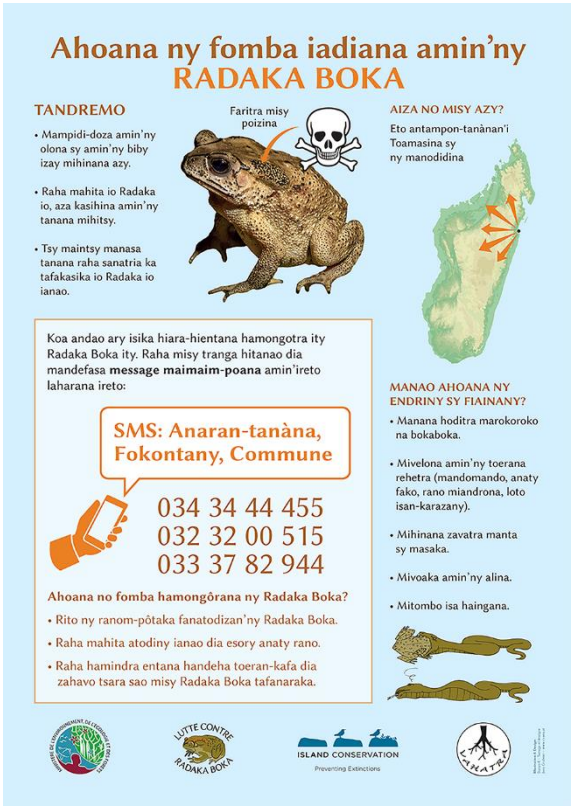

Fig. 2.5 - Poster prepared by Madagascar Fauna and Flora Group during the 'Radaka Boka' awareness campaign

Very close to the incursion area are two highly biodiverse sites: Parc Ivoloina and Betampona Strict Nature Reserve. The former is a 282-hectare forestry station located 15 km north of Toamasina in an area originally covered by lowland rainforest and now dominated by exotic tree plantations, marshlands, rice paddies, lakes and a small patch of native lowland forest (Ramasindrazana 2009) and hosts a moderately rich amphibian community (Crottini et al. 2014b). The Asian toad has apparently not yet (May 2019) arrived there but it is currently reported to be just 300 m from the southern border of the park (R. Mahasoa pers. comm.), so its arrival is likely to be imminent. We believe that Ivoloina is a suitable site for a pilot study to test containment or protective measures that can be later applied to other sensitive areas. Here, MFG is currently working to create a pilot exclusion zone to protect Parc Ivoloina and to learn about the impacts of the toad on the native wildlife in the event of an invasion. Betampona Strict Nature Reserve, located 40 km north-west of Toamasina, is a 2228-hectare forest patch of lowland rainforest surrounded by agriculture and degraded land. It is extremely biodiverse (e.g., Rosa et al., 2012) and is one of the conservation priorities of the area and, indeed, of the country as a whole. Assuming a spread rate of the Asian toad of 3.3 km/year, according to the most-recent estimates (Licata et al. 2019), Betampona will most likely be reached by this invasion within the next decade if nothing is done to arrest its spread, and the Ankeniheny-Zahamena corridor, one of the largest blocks of rainforest in Madagascar, located at a distance less than 50 km from the current front of the invasion, is likely to undergo a similar fate.

Almost 6 years have passed since the first detection of this invasive population of *D. melanostictus* in Madagascar. Many things have been made, but much more needs to be made to try to mitigate this invasion. At present there is the primary need to (1) develop and implement a national biosecurity system, (2) develop an integrated national plan for the long-term management of this biological invasion and (3) establish a small but effective 'National Working Group' whose leadership would be ratified at the national level and whose role should be overseeing the implementation of the national management plan. Efforts should also be invested to facilitate and simplify the administrative procedures to obtain research and collection permits pertaining to urgent time-bounded research, such as is needed when actively trying to engage with an invasive-species incursion. And in the event that the National Working Group becomes a reality, we suggest that the approval of such requests should receive a fast favourable or unfavourable response from the National Working Group to ensure prompt actions can be developed in the field.

The national management plan must be implemented quickly and with an adaptive management approach that ensures that it learns from the successes and failures of its implementation and undergoes regular revision and review to incorporate those learnings into the active strategy.

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Chapter 3

Article 2

Abundance, distribution and spread of the invasive Asian toad *Duttaphrynus melanostictus* in eastern Madagascar

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3.1 Abstract

The Asian toad, *Duttaphrynus melanostictus*, was accidentally introduced to Toamasina (eastern Madagascar) around 2010, and since then has spread at a substantial rate across a larger area. This study documents the expansion of the invasive range of this species, calculates the invasion spread rate, and provides estimates of toad abundance and habitat preferences. Updates of the distribution range revealed a fivefold increase of the invaded area over 3 years, and a doubling of the rate of spread, showing a shift of the invasion towards the North-West, most probably because of the absence of ecological barriers. We used *N*-mixture models to estimate toad abundance on the basis of repeated count data of six areas in Toamasina and its surrounding countryside. Toad distribution shows heterogeneous density across the distribution range, with an average abundance of 184 toads ha^{-1} (95% CI 132–263). The toad's abundance was highest in sites with the presence of organic waste, and was negatively related to the density of road networks in the proximity of

study sites. The rapid expansion of the Asian toad in the Toamasina region suggests that this toad is an increasing threat for Madagascar. We propose immediate management actions that could limit the spread of alien toads in this megadiverse country.

3.2 Introduction

Madagascar is one of the most celebrated biodiversity hotspots (Myers et al. 2000), where long geographical isolation and geological stability have favoured the evolution of a unique biodiversity (Goodman and Benstead 2003; Crottini et al. 2012; Holt et al. 2013; Ganzhorn et al. 2014). Besides the well-known conservation issues resulting from significant environmental degradation (Green and Sussman 1990; Vallan 2002; Harper et al. 2007), in 2014, a series of scientific communications drew attention to a new challenge for nature conservation in Madagascar (Crottini et al. 2014a; Kolby et al. 2014; Andreone 2014; Kull et al. 2015; Moore et al. 2015): an invasive population of the Asian common toad (*Duttaphrynus melanostictus*, hereafter referred to as ‘‘Asian toad’’) was reported in Toamasina, the second city of the country for size and population and the major seaport of the island, located in eastern Madagascar. The ecological similarities with the dramatic invasion history of the cane toad *Rhinella marina*, which determined the decline of multiple endemic vertebrates across Australia (Shine 2010), have raised fears of a similar predicted ecological catastrophe for Madagascar.

The *D. melanostictus* complex is native to South and South East Asia, and has already succeeded in invading multiple regions including Bali, New Guinea, Sulawesi, Timor-Leste and other islands, mostly between Sundaland and Wallacea (Reilly et al. 2017). Recent phylogeographical analyses revealed the Asian toad to be a complex of species, in need of taxonomic revision (Wogan et al. 2016), and the toad lineage identified in Madagascar belongs to the clade distributed in South East Asia. The individuals that first established in Madagascar probably came from an area between Cambodia and Vietnam (Vences et al. 2017).

The Madagascar invasion probably started as early as 2010 (Moore et al. 2015), most likely with the accidental introduction of a few toads via commercial containers from South East Asia (McClelland et al. 2015). After a typical lag phase of a few years, the Asian toad population has grown exponentially, taking advantage of the suitable climate, especially the annual rainy season that likely matches the conditions occurring in the native area of this species (Vences et al. 2017), and directly triggers reproduction in this species (Ngo and Ngo 2013).

Although in its native range this species often lives in disturbed anthropogenic habitats, species distribution models suggested that the Asian toad can potentially spread across the lowlands of the eastern and northern coasts of Madagascar (Pearson 2015; Vences et al. 2017), thus raising the fear that it could threaten native rainforest communities.

The ongoing spread of the Asian toad is likely to pose major threats to the Malagasy native

ecosystems. The most dreaded effect related to this biological invasion is the poisoning of naıve predators (Brown et al. 2016; Marshall et al. 2018). The Asian toads can release a cardiotoxic toxin that can be fatal to predators if ingested (Chen and Kovaříková 1967; Marshall et al. 2018), and the vast majority of native potential predators seems to be non-resistant to bufotoxins (Marshall et al. 2018). Other potential negative effects include the transmission of pathogens to native amphibian species, and the competition for breeding sites and feeding resources (Mahapatra et al. 2017), given its explosive breeding behaviour (Fan et al. 2013) and the sizeable clutches of up to ten thousand eggs (own unpublished data). Currently, published information on the Asian toad distribution in Madagascar has been based on data collected in 2014, when the Asian toad distribution range covered an area of about 108 km^2 across Toamasina and its immediate vicinities (Moore et al. 2015), with maximum densities (of up to 51 toads per 100 m^2) reported for the urban area (McClelland et al. 2015). A more recent report provided wide abundance estimates across different habitats, with average densities ranging from 325 to 987 toads per hectare, and the highest value recorded in urban areas (1800 toads/ha; Reardon et al. 2018).

Although extremely useful to have a first impression of this invasion, this estimate was based on extrapolated simplistic counting approaches, which did not take into account the detection probability of the species. It is therefore necessary to apply a more robust statistical approach to assess overall abundance of the species and update its distribution.

With this study we provide an update on the distribution, abundance and characterize the invasion spread of the Asian toad in the Toamasina Province. Updating the invaded range and the collection of accurate presence–absence data will enable the implementation of robust predictive models of potential distribution of the species. Integrating these data with estimates of abundance across different invaded environments (which also depends on the carrying capacity of the colonised habitats) can further enable the identification of factors determining species densities, and provide information on the type of habitat preferentially selected (or avoided) by the species. Finally, the spread capacity of the invasive species needs to be characterized as it may change across time and space due to the influence of topographic landscape features or due to the evolution of specific dispersal and life-history traits (Perkins et al. 2013). All these different types of information could be used to better assess the risk that this invasive species is posing to native communities. This information is also critical to inform the implementation of delimitation and containment measures (Stohlgren and Schnase 2006), which will be fundamental to any effort to prevent the spread of Asian toads into ecologically sensitive areas or for attempting eradication of smaller satellite populations.

3.3 Materials and methods

3.3.1 Study area

To update the distribution map and assess the invasion spread, broad scale monitoring was performed, trying to cover the whole Province of Toamasina (Toamasina I, Toamasina II, and Brickaville Districts). The northernmost locality visited was Mahavelona (60 km from Toamasina), while the southernmost was Brickaville (80 km from Toamasina) (Fig. 3.1a).

We opportunistically selected 6 localities where the toad was detected to estimate species abundance and relationships between abundance and environmental features (Fig. 3.1b). The chosen sites represented the two main habitat types where the species is currently reported: (a) urban and (b) agricultural/seminatural localities (sensu Moore et al. 2015). The three urban localities are within the border of the city of Toamasina: district of Mangarano (18°80'46'' S 49°22'47'' E), Amparihilava (18°10'7''S 49°22'7''E), and Barikadimy Campus of the University of Toamasina (18°7'49''S 49°22'42''E). Two localities predominantly associated with an agricultural landscape are located in Farafaty Village (18°80'33''S 49°21'27''E) and in Ambodibonara Village (18°80'21''S 49°20'30''E). Here the area is characterized by the presence of rice paddies, mixed fruit trees, sugar cane plantations, and surrounded by degraded forest and mixed shrubland (locally called ''savoka''). The last locality is a palm oil plantation located at about 10 km South of Toamasina, next to the village of Mahasoa (18°13'51''S 49°18'18''E).

At the time of the first delimitation survey that took place in 2014 (Moore et al. 2015), both Mahasoa and Ambodibonara were located at the fore front of the toad invasion area.

Farafaty was selected among the sites that were used to test the efficiency of different control methods (Reardon et al. 2018). These trials took place between mid-January and April 2016, and were conducted for the purpose of assessing the efficacy of potential eradication tools to better understand the technical feasibility of Asian toad eradication.

3.3.2 Invaded area and dispersal rate

A total of 104 localities were inspected between November 2016 and September 2017 (Table B.1), reporting the presence or non-detection of toads. Coordinates were recorded by means of a Garmin 60CSx GPS device. Localities were selected opportunistically, based on received notifications of possible toad occurrences to the toll-free hotline number instituted by the local NGO ''Madagascar Fauna and Flora Group'' (MFG), and through selection of sites that were outside the recorded known distribution of the toad in 2014 (Moore et al. 2015), that were chosen to identify the invasion front and carry out MFG's awareness-raising activities concerning the toad. Upon arrival, a field crew of ca. ten people conducted interviews with local residents, followed by visual encounter surveys (VES) that took place after dusk (from 18:00 to 21:00) (as per Moore et al. 2015). The invaded area was evaluated by calculating the minimum convex polygon (Worton 1987), using the convex hull tool of ArcGIS 10.2 (ESRI 2011). To estimate the maximum invasion rate, we considered

the maximum linear distance between the presumed introduction point (i.e., the centroid calculated

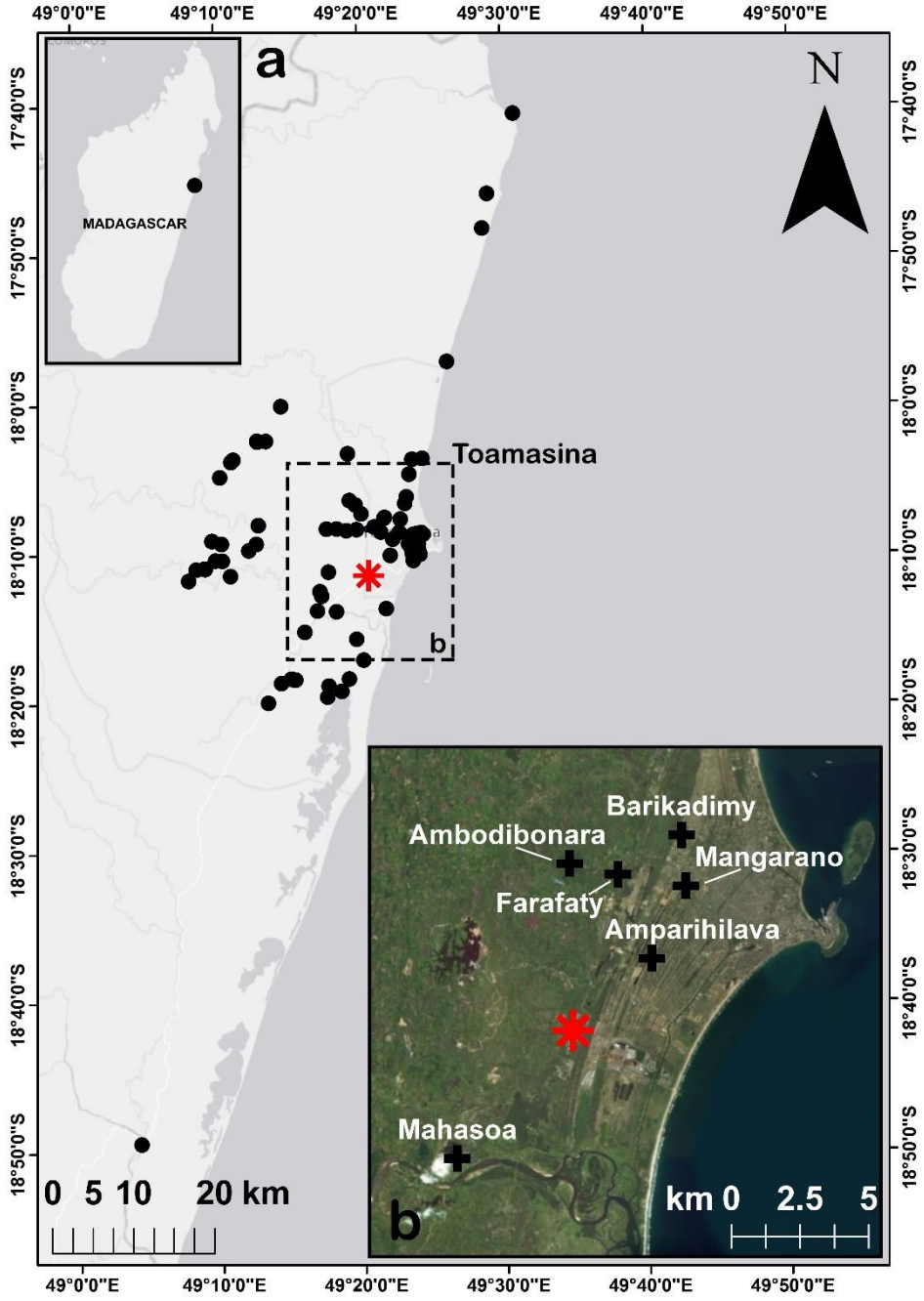

Fig. 3.1 - a) Location of study area with sites visited (filled circle) and the presumed introduction point calculated for data collected in 2014 (star; Moore et al. 2015) to update the distribution range of the Asian toad in Toamasina (Madagascar). The map also shows the positions of Parc Ivoloina and Betampona Strict Nature Reserve, which is the protected area nearest to the Asian toad invasive range; (b) sites selected for the study on toad abundance (greek crosses) (December 2016–January 2017).

in 2014 ; Moore et al. 2015) and the most distant location recorded, representing the invasion front, divided by the time difference between the date of first detection in that locality and the estimated start date of the invasion. We arbitrarily chose mid-May 2010 as the start date of the invasion. This, represents the first reported estimate of the first toad observations in Toamasina (Moore et al. 2015). We assumed one single introduction event, as suggested by genetic data (Vences et al. 2017). We calculated the mean spread rate by dividing the distances between all invasion front localities and

the presumed introduction point by the time difference between the date of first detection of the toad in the locality and the presumed start date of the invasion, and averaging the results. This datum was then compared with the data available from the study of Moore et al. (2015).

3.3.3 Estimation of abundance and environmental features

The study sites were spaced at least 1 km apart from each other. Within each locality we selected five quadrat plots (20 x 20 m), spaced at least 100 m from each other. Authorization was sought from residents and landowners prior to establishing plots. Each plot was individually marked with plastic flags and geo- referenced. Thirty quadrat plots were established in total, covering an area of 12,000 m^2.

From 12th of December 2016 to 21st of January 2017, two experienced observers visited each site four times at regular time intervals (7–8 days), starting the counting soon after dusk (18:00–21:00), when toads are most active. Each count took approximately 10 min to inspect the whole plot area; detected toads were not removed. For each quadrat plot, we measured four environmental variables: *Road*, the distance from the nearest road, with plots including roads to investigate the effect on toad abundances, which could be both positive (Brown et al. 2006) or negative (Fahrig et al. 1995; Andrews et al. 2009); *Buildings*, the number of buildings within a radius of 100 m; *Tree*, the percentage of tree cover within a radius of 100 m; *Waste*, the presence of organic waste (i.e. household rubbish or agricultural waste) within the plot or along its borders was noted. Organic waste often shows high arthropod abundance (Frankie and Ehler 1978; McIntyre 2000), and can represent foraging hotspots for toads, which are typically associated with anthropogenically degraded habitats (van Dijk et al. 2004).

To determine building density and the percentage of tree canopy cover, we analysed Google Earth satellite imagery of 2017 using the software imageJ (as per Ricotta et al. 2014). Two weather conditions were obtained from the local meteorological station, located at Toamasina airport: precipitation during the 24 h before the survey (mm) and temperature at the time of the survey.

3.3.4 Statistical analysis

We used *N*-mixture models to estimate toad abundance on the basis of repeated count data, and to identify the environmental variables determining variation of abundance. This class of models allows estimation of animal abundance from repeated surveys at fixed sites, without marking individuals to identify them (Royle 2004). Some studies highlighted that the joint estimation of abundance and detection probability can be problematic, and that these models are sensitive to violations of their assumptions (Barker et al. 2018; Duarte et al. 2018). Nevertheless, analyses of real-world data suggested that this approach can provide reliable estimates and can be extremely

useful to measure the abundance of wild animals (Kéry 2018; Ficetola et al. 2018a).

Our sampling was performed during 40 days, thus we assumed that populations were closed. *N*-mixture models were fitted using a Zero-Inflated Poisson error distribution (ZIP), as ZIP models consistently showed lower Akaike's Information Criterion (i.e., AIC_c, Burnham and Anderson 2002) than Poisson models. Negative binomial models were not considered because of their convergence issues (Kéry 2018). In *N*-mixture models, the upper bound to approximate an infinite summation in the likelihood was ''100 + the maximum observed species abundance'', as preliminary analyses and simulations suggest that this value provides robust and stable estimates (Ficetola et al. 2018b).

We built models including weather conditions as variables influencing toad detection, and environmental variables of sites as potential predictors of toad abundance. We built models including all potential combinations of independent variables, ranking them on the basis of their AIC_c values, using a model selection approach that excludes from the confidence set the overly complex models (Richards et al. 2011). Models with a $\Delta AIC_c > 10$ were excluded from candidate model set (Burnham and Anderson 2002). Spearman's rank correlation coefficient for site covariates indicated lack of significant correlation among the ecological parameters considered, allowing us to use all covariates in the model building procedure.

Data were analysed using the software R (version 3.5.0; R Core Team 2018) with the package Unmarked (Fiske and Chandler 2011) and AICcmodavg, to assess the strength of the different models tested (Mazerolle 2011). We used the package spdep (Bivand et al. 2005) to assess the spatial autocorrelation of the residuals of the global model.

The results of this analysis suggested the absence of spatial autocorrelation (Moran's *I* statistics = - 0.03, $P = 0.48$).

3.4 Results

3.4.1 Distribution

The Asian toad was detected in 50 out of 104 visited localities (Table B.1), resulting in a minimum convex polygon of 549 km^2 (Fig. 3.2). The current centroid is located 6.75 km and 295° North-West with respect to the last calculated centroid (Moore et al. 2015).

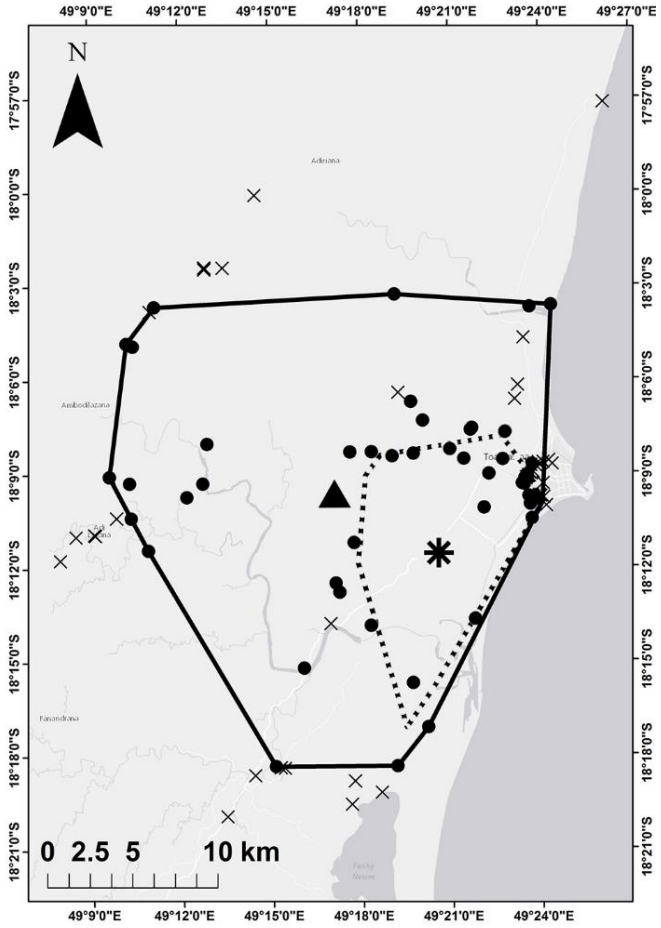

Fig. 3.2 - Map of the current (early 2017) distribution range (solid line) of Asian toad showing sites of detection (filled circles) and non-detection (crosses). The dotted line indicates the 2014 distribution range (Moore et al. 2015). The filled triangle represents the current distribution centroid, located 6.75 km W of the last calculated centroid (star; Moore et al. 2015)

The maximum distance between the putative introduction point and the furthest location so far recorded is 21.9 km. Assuming that no other introductions have occurred, the maximum spread rate of toads is 3.3 km year^{-1}, while the average spread rate based on all invasion front localities was of 2.5 ± 0.6 km year^{-1} (Fig. 3.3).

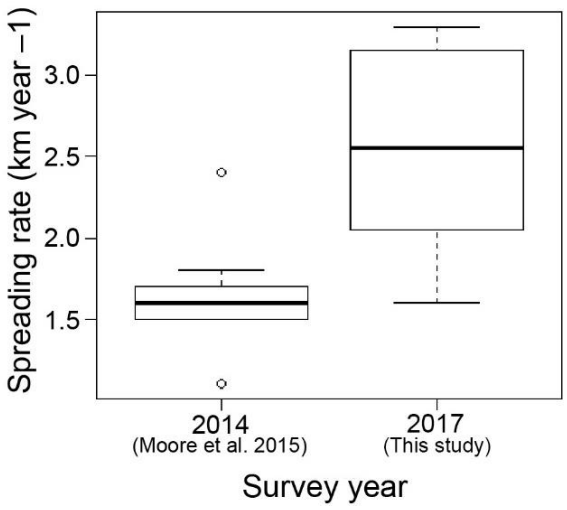

Fig. 3.3 - Box-plot showing the invasion spread rates, comparing the estimates of this study with data available from Moore et al. (2015)

3.4.2 Abundances

During our surveys, we detected 363 Asian toads (range 0–23 individuals per survey per plot). Toads were not detected in 20% of surveyed plots over 4 visits.

The model with zero-inflated Poisson distribution (ZIP) showed the lowest AICc values and was employed as the global model. The best AICc model (Table 3.1) suggested that weather conditions did not influence the detection of toads, and among the environmental covariates, waste presence (*Waste*) and road proximity (*Road*) were the most important variables in describing toad abundance.

Table 3.1 - List of candidate models relating toad abundance to environmental features ranked by AICc values

Models	***df***	**AICc**	**ΔAICc**
r (Survey) *λ* (Waste + Road) [a]	4	411.3	0
r (Survey) λ (Waste + Buildings)	4	432	20.6
r (Survey) λ (Waste + Tree)	4	477.5	66.2
r (Survey) λ (Waste)	3	479.7	68.3
Null model	2	503.7	92.3

[a]Best model describing Asian toad abundances

Toad densities were highest in sites with more organic waste (B = 2.93, P < 0.001), which was particularly abundant in urban and agricultural degraded areas with open-air dumps. Conversely, road proximity had a negative relationship with toad abundance (B = 0.44, P < 0.001). The estimated per-individual detection probability was 0.455 ± 0.04 (mean ± SE), which corresponded to a site-level detection probability ranging between 0.62 and > 0.99, depending on species abundance. The estimated mean abundance per plot was 7.4 individuals (median = 4.2; 95% CI 5.3–10.5), with estimated numbers of individuals per plot ranging from 0 to 59. The total abundance across the 30 study plots (total surface: 12,000 m^2) was 221 individuals (95% CI 158–316), which means a density of 184 toads ha^{-1} (95% CI 132–263). The abundance showed strong variation across the surveyed sites, and no strong differences were detected between urban and seminatural localities (Fig. 3.4). The highest abundances were recorded at the palm oil plantation (Mahasoa, see Fig. 3.4), where the estimated abundance was 559 toads ha^{-1} (95% CI 445–695).

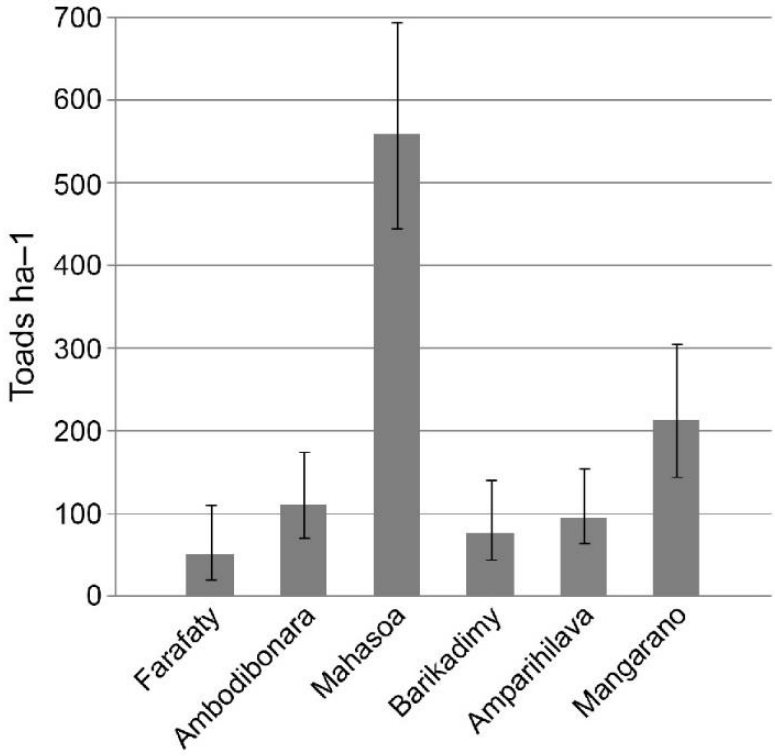

Fig. 3.4 - Histogram of toad abundances across surveyed sites. Lines represent 95% confidence intervals.

3.5 Discussion

Although Asian toads have successfully invaded several regions of the world, there is a general lack of studies on the ecology of this species (Reilly et al. 2017). Our work provides a detailed analysis of the invasive potential of the Asian toad in eastern Madagascar, updating information on the ongoing invasive process and identifying the habitat features related to the variation of species abundance.

3.5.1 Rate and patterns of invasion

Our study highlights a rapid advancement of the invasion front, with an expansion of the toad particularly towards the North-West (Fig. 3.2), probably due to the absence of ecological barriers (Brown et al. 2015). Conversely, the northward toad invasion has been probably slowed down by the presence of the River Ivoloina, suggesting that this river functioned as a partial barrier, as already reported in literature (Leblois et al. 2000; Li et al. 2009; Zhao et al. 2009). Contrary to this, the river Ivondro in the South does not seem to play the same role in arresting the invasion; this might be due to the presence of multiple man-made canals and waterways, primarily used for transportation and fishing, that could have further facilitated the invasion, increasing the connectivity between previously isolated biota (Crooks and Suarez 2006; Rahel 2007). Nonetheless, due to the lack of a rigorously defined sampling design for assessing the invasive range, our results may be subject to spatial and

detection biases. It should, therefore, be noted that the current distribution update is not exhaustive and is likely to be very conservative.

Our estimate of spread rate (2.5–3.3 km/year) is two-times higher than the previously reported values (Moore et al. 2015) (Fig. 3.3). This may be real acceleration, or it may be the result of an underestimate by Moore et al. (2015). An acceleration of invasion rate has been extensively reported during the invasion of cane toads in Australia, where it has been associated with the rapid adaptive changes in morphological and behavioural traits, and promoted by the absence of ecological pressures (Phillips et al. 2006). Available data suggest that the Asian toad population in Madagascar lives in environments strongly similar to the ones occupied by the species in its native range (Vences et al. 2017). Apart from sporadic killings by local people and some limited population control efforts in very small targeted areas, toads are probably not exposed to strong predation pressure because of their toxicity (Marshall et al. 2018), even though during the initial stages of colonization they could suffer attacks from naive predators. There is an urgent need to verify if the observed increase of the spread rate reported for Madagascar is facilitated by favourable local environmental conditions (as observed in Australia with cane toads; Urban et al. 2008), or if it is related to human-mediated introductions to new regions. The monitoring of domestic trade of building mate- rials (e.g., concrete blocks or wood piles) and the movement of agricultural products (e.g., palm oil empty fruit bunches) should be prioritized, since these substrates are often used as shelters by the Asian toads (FL, pers. obs.) and human-mediated movement of these goods could inadvertently contribute to the species range expansions.

3.5.2 Abundance and habitat preferences

Abundance is a major factor determining the impact of invasive species (Leung et al. 2012), and identifying the influencing factors is relevant to understanding the causes of its spread and persistence in the invaded area. The mean abundance herein reported finds both discrepancies and similarities with data available from other invasive amphibian species. Studies on *Rhinella marina* showed that populations can reach very high densities in newly colonized habitats (> 2000 toads ha^{-1}, (Freeland 1986), probably because of high prey availability and reduced competition with conspecifics during the first stages of invasion (Brown et al. 2013). Interestingly, in Papua New Guinea, Zug et al. (1975) reported consistent shifts in abundances across different habitats, where 30 cane toads ha^{-1} could be found in savannahs, whereas in forested areas the number was ten times lower.

Overall, our results showed that the Asian toad shows a strong variation of abundance in Madagascar (Fig. 3.4), and abundances are positively correlated to the presence of rubbish dumps. This relationship is probably due to the high edaphic humidity, the presence of favourable shelters and the high abundance of arthropods, which are the favourite prey (Frankie and Ehler 1978;

McIntyre 2000). Conversely, toad abundance is lowest near to roads. Although roads can be used as dispersal corridors by invasive species (e.g., in cane toad; Brown et al. 2006), in our study sites it seems that roads act as a source of stress for the toad, as already reported in other amphibians (Fahrig et al. 1995; Andrews et al. 2009). Nearby roads, they might be killed by local people more easily due to higher detectability, or might be more exposed to the risk of roadkill.

The relationship between invasive toads and human disturbance has already been observed in the cane toad, where the invasive species actively selects breeding sites where anthropogenic disturbances have occurred. For instance, in the Solomon Islands, cane toads showed densities 3.11 times higher in disturbed (vs. non-disturbed) habitats (Pikacha et al. 2015). In our study, we found no difference in abundance between urban and seminatural sites but interestingly, we found the highest densities at the palm oil plantation locality of Mahasoa (Fig. 3.4), where, in a quadrat plot located nearby an open-air rubbish dump filled with agricultural waste, up to 23 individuals were counted. Here, the carrying capacity could have been increased by local management practices. Indeed, tons of plant remains (e.g., empty fruit bunches) are used as mulch for local plantations (Hamdan et al. 1998). Asian toads often exploit this substrate and it will be important to verify if, during the relocation of the mulch across plantations, toads are also moved into new palm oil parcels.

The relationship between toad abundance and canopy cover should be further investigated, even though no significant relationships were found during this study. The canopy cover percentage obtained by satellite imagery does not provide a quantitative measure of habitat features within the surveyed area, as it overlooks differences between habitats (e.g., differences between plantation vs. secondary forest). Indeed, in several plots located in highly forested areas toads were absent, suggesting that those habitats might have lower suitability, as already reported for several bufonid species that typically prefer poorly vegetated or open areas (Guerry and Hunter Jr. 2002; Tucker and Simmons 2009), but a finer study using radiotelemetry should be applied to better assess the suitability of forested habitats. It should be noted that the ecological range and replication of sites where abundance was investigated in this study remains small and so further replications and sampling of ecological variables are needed to enhance the robustness of our estimates.

3.6 Conclusions

The rapid range expansion of the Asian toad in the Toamasina region confirms that this toad represents an increasing threat for Madagascar (Fig. 3.2), with an observed occupied area at least fivefold larger than that previously estimated in 2014 and an observed doubled spread rate than recorded in the last surveys (Moore et al. 2015). We provide estimates of the abundance of this species across different selected sites. Estimates obtained from the semi-natural site of Farafaty may be slightly lower due to the small-scale eradication trials conducted 8 months prior to this study that might have affected the abundance estimation, even if reinvasion into the control trial areas is

likely to have been occurring in the intermediary period. Finally, the analysis of relationships between toad abundance and environmental variables provides important management indications. The strong correlation between waste presence and toad abundance suggests that dump sites should be prioritized for the implementation of control measures, as proper waste control, management and disposal programs could help in reducing feeding resources and shelters for this invasive species.

Madagascar is one of the most biodiverse countries in the world, and this invasion represents a major threat for its native biodiversity and for the country's economy, which largely depends on wildlife eco-tourism (Duffy 2006). Such rich and unique ecosystems are already threatened by multiple anthropogenic pressures, and biological invasions are rapidly emerging as a major threat to biodiversity and ecological robustness (Crowl et al. 2008; Bellard et al. 2014, 2016), highlighting the need for Madagascar to develop and establish biosecurity protocols.

An eradication plan now seems to be very difficult to implement, given the large scale of the invasion (McClelland et al. 2015; Reardon et al. 2018), worsened by the difficulty to make such activities a priority in a country as economically impoverished as Madagascar. Notwithstanding, it is important to intensify the research on the parameters influencing the biology and habitat occupancy of this species, as this will help inform delimitation and containment protocols for Asian toads in any future mitigation efforts. For example, Parc Ivoloina represents an important amphibian biodiversity stronghold (Crottini et al. 2014b), and lies in close proximity to the city of Toamasina (Fig. 3.1). This site represents a perfect pilot study site to develop containment measures to exclude the toad, and represents an opportunity to develop protective methods that can later be applied at the Betampona Strict Nature Reserve (ca. 30 km north west from Toamasina; Fig. 3.1), an important biodiversity hotspot which hosts an incredibly high number of microendemic frog species (Rosa et al. 2012) that are directly threatened by the continued spread of the Asian toad.

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Chapter 4

Article 3

Does spatial sorting occur in the invasive Asian toad in Madagascar? Insights into the invasion unveiled by morphological analyses

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4.1 Abstract

Dispersal-relevant traits may be subjected to spatial sorting in rapidly expanding populations, driving the evolution of dispersive phenotypes. This process has been largely documented in invasive species, and ascertaining its presence can have important implications for their management. The Asian common toad was accidentally introduced to Madagascar around 2010 and is a highly problematic invasive species. Exploring the morphology of this species offers the twofold opportunity to test the hypothesis of early onset of spatial sorting and to gather information on the dynamics of this unstudied biological invasion. For these purposes, we analyze morphological data of adult toads collected across the invasive range in Madagascar, and we test the effect of the distance from the introduction point on the relative size of head, radioulna and tibiofibula, and on body size and body condition of toads, respectively. We did not detect evidence of spatial sorting in the morphological traits analyzed. Instead, morphological variation was largely dependent on sex and body size of individuals. Sexual dimorphism was limited in relation to other populations in the native range, and body size did not vary across the invasive gradient, which could indicate that both adults and

juveniles are currently contributing to the dispersal in this invasive species. We provide important baseline data for the long-term assessment of morphological variation in the invasive population, and we advocate for further investigations of spatial sorting in life history and behavioral traits.

4.2 Introduction

Individuals at the edges of an expanding population may be subjected to differential selective pressures compared to conspecifics from long-colonized areas, which can possibly lead to the emergence of distinctive phenotypic traits (Chuang and Peterson 2016). This evolutionary mechanism, named spatial sorting, is driven by intrinsic differences of dispersal capabilities among individuals and inevitably brings to the accumulation of dispersal-relevant traits at the leading- edge populations (Shine et al. 2011; Phillips and Perkins 2019). Spatial sorting can positively select a broad range of traits, including life history, behavioral, physiological, and morphological features (Chuang and Peterson 2016).

Although spatial sorting has been documented in naturally expanding populations, where range shifting can be induced by climate change (e.g., Sanford et al. 2006; Hassall et al. 2009), biological invasions represent the best study system for investigating this phenomenon, as invasion speeds can be faster than natural expansions of species ranges. Invasive populations of the cane toad *Rhinella marina* (Linnaeus, 1758) in Australia are an impressive example of such a phenomenon. In this invasive species, spatial sorting has occurred on a whole set of dispersal-related traits. Leading-edge populations expanding in monsoonal floodplain and savannah areas of northern Australia show enhanced dispersal abilities due to several behavioral (Brown et al. 2007; Alford et al. 2009), locomotor, and morphological changes (Phillips et al. 2006; Hudson et al. 2016b, 2020). As a result of spatial sorting, cane toads from these invasion forefronts have proportionally narrower heads, longer tibiofibulas and radioulnas, and better dispersal ability (Phillips et al. 2006; Hudson et al. 2016a, 2018). Furthermore, larger and heavier adult cane toads are leading the invasion forefront, as they have higher dispersal capacity than smaller individuals and juveniles, which arrive months later, revealing clear spatiotemporal patterns of invasion (Brown et al. 2013). Limb proportions show a complex pattern across the whole invasive range, initially decreasing from the central to the intermediate populations, where natural selection promotes short-legged individuals. The trend sharply reverses near the invasion front, where individuals have significantly longer limbs and better dispersal ability (Hudson et al. 2016b; Stuart et al. 2019). This reveals how trade-offs between contrasting evolutionary forces (i.e., natural selection vs. spatial sorting) determine complex patterns of phenotypic variation across populations (Hudson et al. 2016b; Clarke et al. 2019). In addition, environmental features of the recipient ecosystems (Urban et al. 2008) can promote or hinder species dispersal, determining the spatial sorting onset.

Spatial sorting can strongly influence multiple features and the dispersal of invasive populations. Therefore, ascertaining the occurrence of this pattern helps to gather important information on invasion dynamics and to identify better management responses.

The Asian common toad *Duttaphrynus melanostictus* (Schneider, 1799) is a smaller confamilial of the cane toad. Native to Southeast Asia, it was accidentally introduced to Madagascar around 2010 (Moore et al. 2015). The recent invasion of the Asian common toad in Madagascar is posing unprecedented threats to Malagasy biodiversity. First, these toads are toxic and can cause the poisoning of native predators (Marshall et al. 2018). Furthermore, due to its high fecundity, the toad could attain high abundances with multiple potential impacts on native species, especially amphibians, including competition and the transmission of pathogens (Licata et al. 2020). The invaded area is highly suitable for this species (Vences et al. 2017), characterized by the widespread presence of human settlements associated with rice paddies, which are favorable foraging and breeding habitats for the invasive toad (Jørgensen et al. 1986; Licata et al. 2019), that have granted a successful establishment and are likely to ensure optimal conditions for its dispersal, and the consequent onset of spatial sorting. This invasion provides a unique opportunity of analyzing spatial sorting since the earliest phases of the invasion, thus greatly improving our understanding of the drivers and consequences of this process.

Here, we performed the first broad-scale analysis of morphological variation of the Asian toad across the whole invasive range in Madagascar, to characterize dispersal-relevant morphological traits and test the spatial sorting hypothesis. In so doing, we assessed whether there is a relationship between these traits and the distance from the introduction point. Furthermore, we explored invasion dynamics by testing whether size and body condition of adult toads changed with the distance from the introduction point.

4.3 Materials and Methods

4.3.1 Study system

The invasive population of *D. melanostictus* in Madagascar belongs to a species complex of Asian medium-sized toads (Wogan et al. 2016), which have been accidentally introduced by humans to several regions worldwide, and, in particular, in the Wallacea (Reilly et al. 2017; Othman et al. 2020). This terrestrial, lentic-breeding amphibian is an ecological generalist and thrives in human-dominated landscapes (van Dijk et al. 2004). Females reproduce seasonally, producing up to 10000 eggs per clutch (Ngo and Ngo 2013; FL pers. observ.), and, as in most anurans, are larger than males, but size and sexual dimorphism can vary considerably across populations (range of average body

size across populations: females = 58.0–107.1 mm; males = 50.8–90.6 mm; Guo et al. 2019). The invasive population established in Madagascar originates from an area between Vietnam and Cambodia (Vences et al. 2017) and was likely introduced via accidental stowaway in commercial containers around 2010 (Moore et al. 2015). The putative introduction point of the toad was identified close to the SW suburbs of Toamasina (Moore et al. 2015). From here, the species expanded in all landward directions with a spreading rate of 1.6–2.5 km/year and, in 2017, it already invaded an area of 549 km2, with average abundances of 184 toads/ha (Fig. 4.1; Licata et al. 2019). In 2014, when the invasive range was estimated at only 108 km^2 (Moore et al. 2015), the total population abundance was estimated to be already >7 million post-metamorphic individuals (Reardon et al. 2018). The invasive range is located at low-mid elevations (0–500 m a.s.l.) and is intersected by the Ivondro and Ivoloina rivers, on the southern and northern edges of Toamasina, respectively (Fig. 4.1). Apparently, the Ivoloina river could have acted as a partial barrier, temporarily slowing down the northern front of the invasion (Licata et al. 2019). The invaded area is characterized by a human-modified landscape, with more than 1500 villages or small groups of huts scattered throughout the area, and is largely occupied by croplands (Zhang et al. 2020). Uncultivated land is mainly covered by grass-lands and shrubland, with only a few remnant patches of forest (Zhang et al. 2020). Climatic conditions in the invasive range are highly suitable for this species (Pearson 2015; Vences et al. 2017), being characterized by a tropical rainforest climate (Peel et al. 2007), alternating dry (August–November) and wet season (December– July), and monthly temperatures ranging from 21°C (July) to 27°C (February) (Merkel 2021).

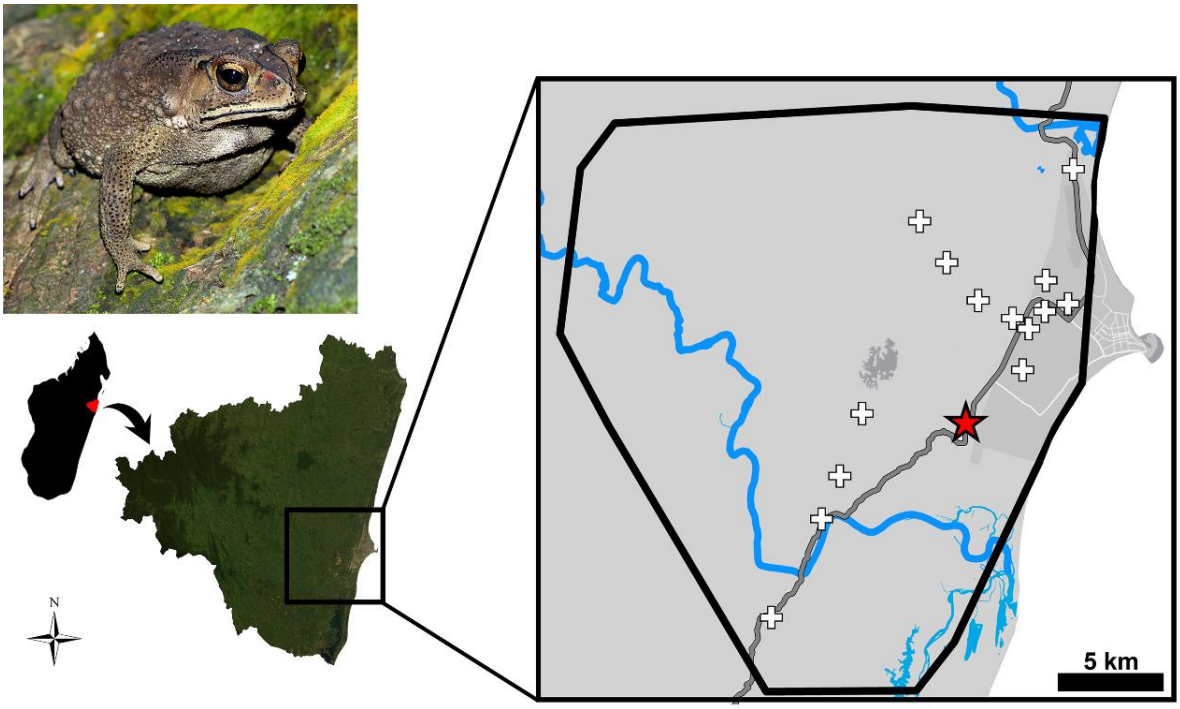

Fig. 4.1 - Map showing the invasive range of the Asian common toad in late 2017 in Madagascar (black line; from Licata et al., 2019) and the sampling localities (white crosses). The red star indicates the proposed site of introduction (Moore et al., 2015), while the dark gray line that crosses it represent the principal route (Route Nationale 2). Photo of Duttaphrynus melanostictus by Franco Andreone.

4.3.2 Sampling

Sampling took place during two different periods (October 2016–January 2017 and October 2018–May 2019), in parallel with other ecological field studies. Localities were chosen opportunistically, mainly along the principal route connecting Toamasina to the capital Antananarivo (Route Nationale 2), which crosses the invasive range from north to south, and also in the urban and suburban districts of Toamasina and in the inland areas of the countryside (Fig. 4.1). We hand-collected adult toads (> 50.2 mm; Ngo and Ngo 2013) encountered during night time, conducting spotlight surveys (visual encounter surveys; Crump & Scott Jr, 1994). We collected the furthermost toads in a locality on the southern invasion frontline along the Route Nationale 2 (18.274°S, 49.254°E). Additional visual encounter surveys in this area, visiting other localities farther south, did not detect any further toad. One of us (F.L.) measured the collected toads using a vernier caliper (±0.1 mm), recording four morphometric parameters: snout–vent length (SVL), head width (HW), tibiofibular length (TBL), and radioulnar length (RUL). All animals were weighed using a digital balance (precision: 0.01 g). Sex determination was based on the external characters, with females larger and heavier than males, which in turn have thicker forearms, nuptial pads and develop an orange gular region (Gordon 1933; Licata et al. 2020).

4.3.3 Statistical analyses

We calculated the body condition of each toad using the Residual Index (RI), which is considered a reliable body condition index (Labocha et al. 2014), and is obtained by extracting the residuals of the regression of mass against SVL of all individuals (both ln-transformed). We ln-transformed all continuous variables prior to analyses, to improve normality and reduce skewness.

We used simple linear, second-, third-, and fourth-order polynomial regressions to check for allomorphic relations between morphological traits (HW, TBL, and RUL) and SVL, including the effect of sex. We used the Akaike's Information Criterion to identify the curve with the best fit (i.e., AIC; Burnham and Anderson 2002).

We performed linear mixed-effect models (LMMs) to test the spatial sorting hypothesis, by assessing relationships between morphometric features and distance from the introduction point. First, we tested the relationship between SVL and distance from the introduction point, while taking into account differences between sexes. Second, we used TBL, RUL, and HW as dependent variables and distance from the introduction point as an independent variable, while taking into account the effect of sex and including SVL as a covariate, to correct for the size effect. For graphical purposes, we plotted the relative TBL, RUL, and HW as percentages, by dividing these measures by SVL and multiplying by 100. Finally, we assessed the variation of RI. In this case, the independent

variables included distance from the introduction point, SVL, sex, day of the year and its quadratic term, to take into account potential seasonal variation in body condition. In all the mixed models, we considered the year of sampling as a random effect to take into account possible variation through time.

We assessed the spatial autocorrelation of the residuals of our models using a Moran's I test (Legendre 1993). Lastly, we calculated the sexual dimorphism index (SDI), following the formula proposed by Lovich and Gibbons (1992) (i.e., SDI = [(Larger sex/ Smaller sex) – 1].

We conducted all the analyses in R environment (version 4.0.3; R Core Team 2020) and made publicly available the code used (http://doi.org/10.5281/zenodo.4779685). Linear-mixed models were performed using the package lme4 (Bates et al. 2015) and test statistics were obtained using the lmerTest package (Kuznetsova et al. 2017), while spatial autocorrelation analysis was conducted using the ecoGenetics package (Roser et al. 2017). All graphics were obtained using the package ggplot2 (Wickham 2011).

4.4 Results

We measured 228 adult toads (89 males and 139 females), captured at distances of 3.7–13 km from the putative introduction point (Moore et al., 2015; Fig. 4.1). Morphology of adult toads varied significantly between sexes (Fig. 4.2), with females larger than males for both total length and all the other morphometric parameters (Fig. 4.2, Table B.1). Males showed proportionally longer radioulnas and tibiofibulas than females, which, however, showed slightly bigger head proportions (Figure 4.2; Table B.1). Overall, the sexual dimorphic index SDI was 0.120.

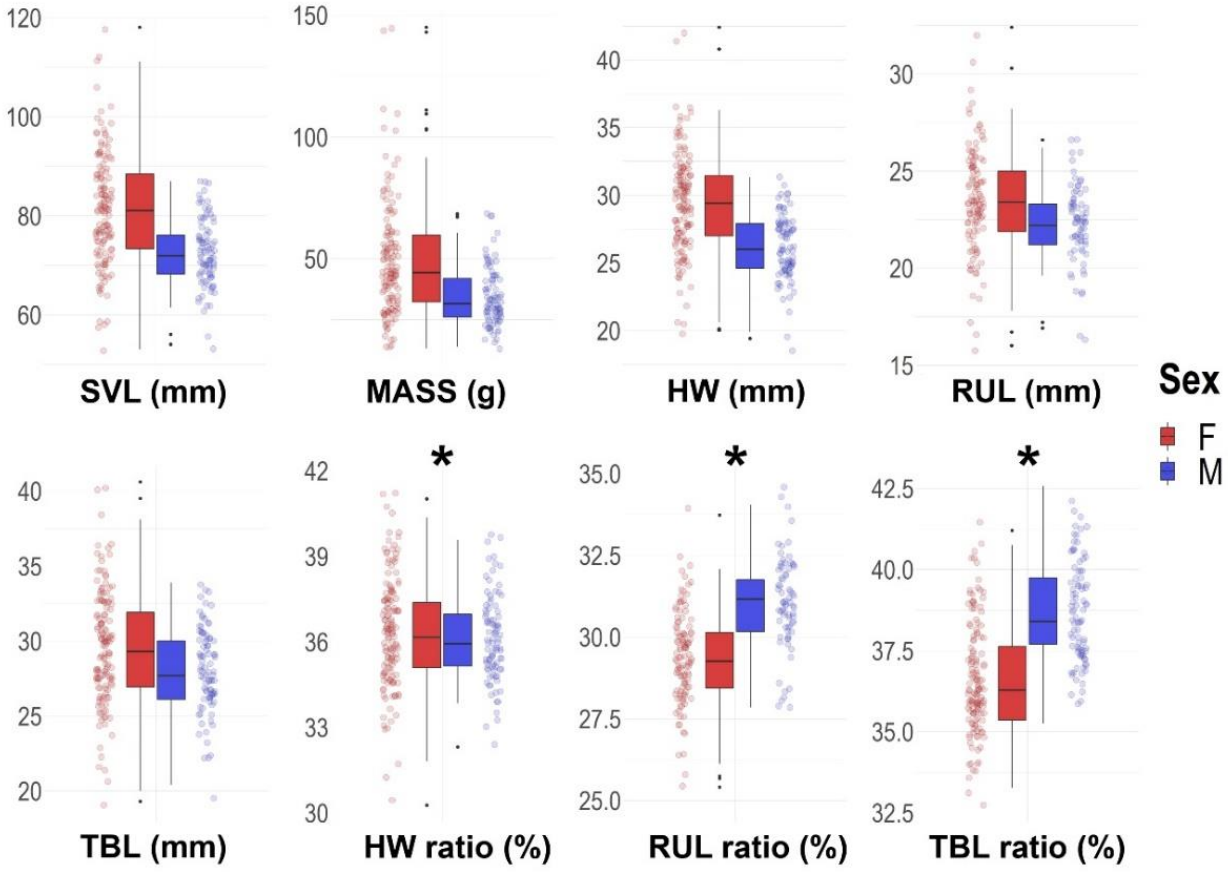

Fig. 4.2 - Boxplots showing differences between toad sexes for the morphological traits analyzed. HW, head width; RUL, radioulnar length; SVL, snout–vent length; TBL, tibiofibular length.

All the relationships between SVL and the other morphometric parameters showed a significant non-linear pattern (Fig. 4.3; Table B.2). The relationship between SVL and TBL was best described by a second-order polynomial curve (adjusted $R^2 = 0.893$), indicating a deceleration of the relationship between SVL and TBL in larger individuals (Fig. 4.3). The relationships between SVL and the other morphological traits were best fitted by a third-order polynomial curve (adjusted R^2: HW =0.891; RUL =0.863), which indicate complex changes in trait proportions during toad ontogenesis (Fig. 4.3).

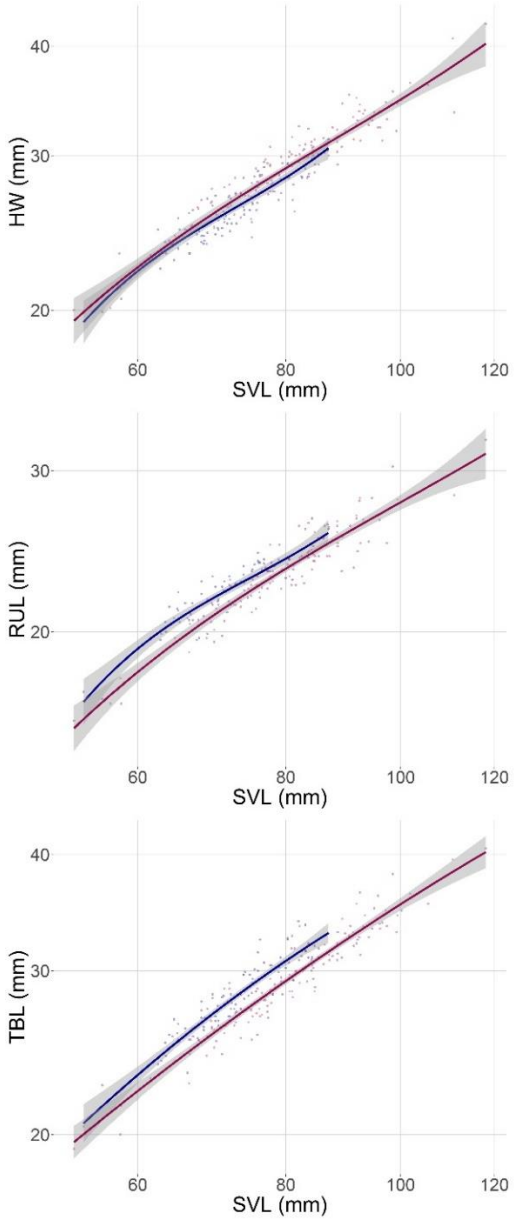

Fig. 4.3 - Polynomial curves fitted to the relation between morphological traits and SVL of the Asian common toads of Madagascar, divided by sex (females in purple and males in blue).

The distance from the introduction point was unrelated to any of the morphological traits taken into consideration, with variation mostly attributable to the sex and/or the size of individuals (see Fig. 4.4; Table 4.1). RI showed a significant linear relationship with the day of the year, indicating an increase of body conditions toward the end of the dry season.

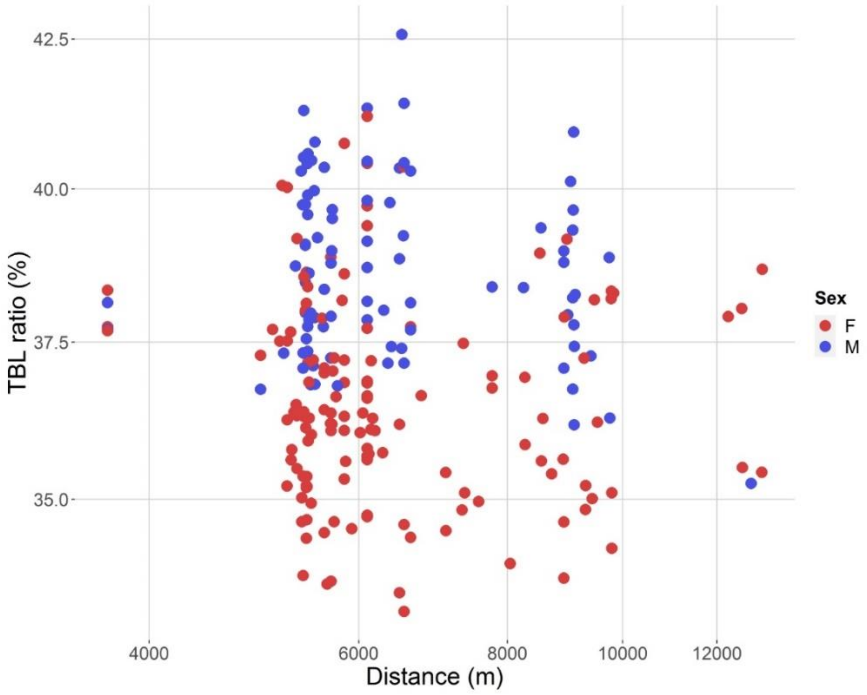

Fig. 4.4 - Relative tibiofibular length (TBLratio) of the invasive Asian common toad of Madagascar in relation to the distance from the introduction point. Sexes are indicated by different colors (females in purple and males in blue).

We did not detect spatial autocorrelation in the residuals of the models with SVL or relative size of head, radioulna and tibiofibula (for all the models, Moran's I < 0.1; $p > 0.1$). Conversely, the residuals of the model with RI as a dependent variable showed significant autocorrelation at lags up to 1.3 km (Moran's I = 0.13; $p = 0.01$). This indicates that, after taking into account the effect of sex and SVL, toads living in nearby areas have similar body condition values.

4.5 Discussion

Dispersal-related morphological traits of the Asian common toad in Madagascar apparently did not change with distance from the introduction point, but were mostly related to the sex and/or ontogenetic phase of individuals, suggesting that spatial sorting is currently not affecting the features of this invasive population. Average body size of adult toads and body conditions did not vary significantly nearby the invasion forefront (see Table 4.1), unlike what has been documented at the cane toad northern invasion forefront in Australia, where larger adults can be found during the first months following toad arrival (Phillips and Shine 2005; Brown et al. 2013). In fact, juvenile toads were also found to be extremely abundant at the invasion front in Madagascar, often outnumbering adults (F.L., pers. obs.), suggesting that they are currently contributing to the range expansion. Further data collection at invasion front localities is required, as well as studies on toad abundances and dispersal abilities.

Table 4.1 - Results of linear mixed models assessing factors determining toads' morphological features.

Trait	Variable	β	d.f.	*t*-value	*p*-value
SVL	Sex F	-0.107	1,224	-6.491	***<0.001***
	Distance	0.034	1,225	0.99	*0.323*
RI	SVL	-0.015	1,217	-0.179	*0.858*
	Sex F	0.025	1,217	1.146	*0.253*
	Distance	0.026	1,217	0.603	*0.547*
	Day	0.413	1,217	2.766	***0.006***
	Day^2	-0.12	1,149	-0.672	*0.503*
TBL	SVL	0.868	1,223	41.567	***<0.001***
	Sex M	0.044	1,223	7.942	***<0.001***
	Distance	-0.007	1,223	-0.695	*0.488*
RUL	SVL	0.787	1,181	32.612	***<0.001***
	Sex M	0.035	1,181	5.683	***<0.001***
	Distance	0.011	1,181	0.921	*0.358*
HW	SVL	0.818	1,222	37.423	***<0.001***
	Sex M	-0.021	1,224	-3.523	***<0.001***
	Distance	-0.012	1,222	-1.085	*0.279*

Significant p-values are highlighted in bold. Abbreviations: HW, head width; RI, residual index; RUL, radioulnar length; SVL, snout-to-vent length; TBL, tibiofibular length.

After taking into account the distance from the introduction point, the body condition index showed a significant spatial autocorrelation at lags up to 1.3 km, suggesting that it is affected by spatially structured environmental features (e.g., food resources) not included in our analyses. Future studies should also include the effect of habitat type on the body condition of the invasive toad, as native populations exhibit lower body condition in human-modified habitats compared with forested habitats (Karraker et al. 2018).

Furthermore, toads were significantly heavier toward the end of the year (see Table 4.1), namely at the end of the dry season. In its native range in central Vietnam, *D. melanostictus* shows reproductive seasonality, breeding mainly between April and July (i.e., during the auxiliary rainy season; Ngo and Ngo 2013), when its body condition decreases (Church 1960). The variation of body condition index could indicate a better foraging activity before the breeding season and/or gonadal maturation, but more analyses are required to clarify the breeding phenology of this species in Madagascar.

In the study population, females were significantly larger than males which, instead, had proportionally longer limbs (Fig. 4.2), a common pattern in toads and other amphibians (Wells 2010). The marked female-biased sexual dimorphism in body size is well known in this and several other toad species (Wells 2010; Ngo and Ngo 2013; Guo et al. 2019). The observed sexual dimorphism index of the invasive toad of Madagascar (SDI = 0.12) stands among the lowest recorded for this species, where the median is 0.201 (range = −0.033– 0.353; Guo et al. 2019). This is due to the larger average size of males in Madagascar (SVL = 72.5 mm; Table B.1) with respect to native

populations (SVL = 66.4 mm; Guo et al. 2019), whereas females are only slightly bigger than the native ones (SVL = 81.1 vs. 79.9 mm; Table B.1; Guo et al. 2019). Variation of sexual size dimorphism could be determined by age differences between sexes (Liao et al. 2013). Further studies will be needed to verify whether the low SDI could be linked to altered selective pressures, which could determine an increased survival rate of adult males, reducing the size gap between sexes and, consequently, sexual size dimorphism.

The lack of spatial sorting in the dispersal-relevant traits considered is likely attributable to the short invasion history of the Asian common toad in Madagascar. *Duttaphrynus melanostictus* was introduced approx. ten years before the beginning of our field activities. In its native range, the Asian common toad apparently attains sexual maturity during the second year of life (Nayak et al. 2007). In Madagascar, this process could be faster, as preliminary skeletochronological analysis suggested a continuous physiological growth of individuals (F.M. Guarino, pers. Comm.), contrary to native populations where lines of arrested growth are observed (Nayak et al. 2007). Preliminary estimates reported a rate of invasion spread ranging between 1.6 and 2.5 km/year (Licata et al. 2019), which is coherent with the annual dispersal rate obtained from radiotracking data on adult toads (approx. 2 km/year; F. Licata, unpublished data), therefore, invasion front toads could be at most from the fifth or sixth generation. Simulation studies predict that conditions at the leading edges of an expanding population can maximize the allelic expression of genes which enhance the spatiotemporal fitness of individuals in approx. 10–15 generations (Phillips and Perkins 2019). In fact, rapid morphological changes resulting from spatial sorting have still not been documented in other invasive species with similar short invasion histories (see Brownscombe and Fox 2012; Merwin 2019), while studies of different species indicate that rapid changes are more likely to emerge in dispersal behavior rather than external morphology (see Lombaert et al. 2014; Ochocki and Miller 2017; Merwin 2019). Now, it will be important to test the occurrence of spatial sorting for behavioral or life history traits, for instance through telemetric studies, laboratory experiments, or common-garden breeding experiments, which allow investigating evolutionary changes by reducing the potential environmental influence of maternal effect. Furthermore, morphological analysis following a systematic random sampling design (e.g., SECR; Royle et al. 2013), targeting discrete and independent localities, could complement such studies and allow ascertaining the lack of spatial patterns in the morphometric traits of the invasive population.

The study of biological invasions is a key source of information on contemporary evolution (Reznick et al. 2019; Melotto et al. 2020). The implementation of long-term studies of the Asian common toad in Madagascar will allow monitoring of variation of its populations during the invasion, providing a great opportunity to understand the evolutionary dynamics of invasive species. In turn, understanding multiple processes occurring during invasion dynamics is essential for informed management strategies.

4.6 Acknowledgments

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Chapter 5

Article 4

Insights into invasion dynamics of the Asian common toad in Madagascar from spatial behavioural analyses

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5.1 Abstract

Invasion dynamics are determined, among other aspects, by the spatial behaviour of invasive populations. The invasive toad *Duttaphrynus melanostictus* is spreading inland from the eastern coast of Madagascar, causing considerable impacts. Understanding the factors determining the spread dynamics can inform management strategies and investigate spatial evolutionary processes. We radio-tracked 91 adult toads in three localities along the invasion gradient to determine whether spatial sorting of dispersive phenotypes is occurring, and investigate intrinsic and extrinsic determinants of spatial behaviour. Overall, toads in our study were habitat generalists, with sheltering behaviour tied to water availability. We did not detect any spatial sorting of dispersal-relevant traits nor sex-biased dispersal, although males moved shorted distances than females and stayed closer

to permanent water bodies, may be due to the prolonged breeding strategy of this species. Additionally, toads had low displacement rates (mean = 4 m/day) and quite philopatric behaviour, but were able to perform long-distance movements, and travelled longer distances under wet weather conditions. Our results also suggest that toads are more likely to expand their range during the wet season, and that the range expansion is probably dominated by short-distance dispersal at this stage of the invasion, although a future increase in invasion speed is expected, due to the long-distance dispersal potential of this species.

5.2 Introduction

The spread dynamics of invasive alien species (hereafter: invasion dynamics) represent the complex outcome of the interaction between population dynamics, biotic and abiotic features of the recipient environments (Hui & Richardson 2017). Dispersal (i.e., the unidirectional movements of individuals towards or between breeding sites; Clobert et al. 2012) plays a prominent role in defining invasion dynamics, and can determine clearly distinct patterns. For instance, invasive populations usually exhibit exponential range expansion following a process of niche filling, while in dispersal-limited species, this pattern may turn linear with a constant rate of spread (Hui & Richardson 2017). Conversely, a stratified dispersal of the invasive population (i.e. when both short- and long-distance dispersers contribute to the range expansion; Shigesada et al. 1995) may lead to an initial slow linear expansion, followed by a boosting phase determined by the coalescence of satellite colonies established beyond the range border by long-distance dispersers (Hui & Richardson 2017). A similar accelerating pattern can be driven by spatial sorting of dispersive phenotypes at the invasion front, where dispersal-relevant traits are positively selected increasing dispersal capabilities and accelerating the spread rate of the invasion through generations (Chuang & Peterson 2016; Hui & Richardson 2017).

Identifying the mechanisms underlying the spread of invasive alien species is key to forecasting and managing biological invasions, but implies understanding of the dispersal dynamics of the target species. This requires specific information on ongoing processes in invaded ranges, as dispersal is strongly context-dependent (Cayuela et al. 2020).

The Asian common toad (*Duttaphrynus melanostictus*) is a species complex of medium-sized toads native to South and South East Asia (Wogan et al. 2016), which established several invasive populations in multiple localities (Reilly et al. 2017) and is considered an emerging, highly problematic invasive species (Measey et al. 2016). The recent introduction of this invasive toad in Madagascar —a global hotspot of biodiversity (Conservation International 2019)— is particularly worrying due to its toxicity and the lack of resistance to toad toxins among native vertebrate predators (Marshall et al. 2018), with at least one species already suffering significant impacts (Licata et al. 2022). The invasive population has spread over a large area (>500 km^2; Licata et al. 2019) and is

deemed to be ineradicable with current management methods (McClelland et al. 2015). Understanding the invasion dynamics of this species and identifying the major factors driving its dispersal is pivotal to predicting the areas suffering the highest invasion risk in the next years, and to plan management actions aimed at limiting its spread and mitigating its impacts.

Toads exhibit the longest dispersal distances amongst anurans, however, these may substantially differ between and within species (Smith & Green 2005; Cayuela et al. 2020). An outstanding example is the invasive cane toad (*Rhinella marina*) in Australia, where populations at the invasion front exhibit a fivefold higher rate of dispersal due to spatial sorting of dispersal-relevant traits (Shine et al. 2021).

Both theoretical and empirical studies suggest that spatial sorting is more likely to emerge in behavioural traits rather than external morphology (Myles-Gonzalez et al. 2015; Van Petegem et al. 2015). The invasive Asian common toad in Madagascar does not show spatial sorting of morphological traits (Licata et al. 2021), and investigating its spatial behaviour offers the opportunity to explore the evolutionary dynamics underlying the onset of spatial sorting in the early stages of a biological invasion. However, the straightforward identification of spatial sorting of behavioural traits may be hindered by a multitude of intrinsic and extrinsic factors which regulate toads' spatial ecology. For instance, desiccation risk can influence daily movement rates and spatial ethology, which largely depends on thermal and moisture conditions (Schwarzkopf & Alford 1996; Brown et al. 2011; Madelaire et al. 2020). Dispersal may also be determined by toad breeding phenology, which is dictated by rainfall regime and water availability (Duellman & Trueb 1994). Furthermore, toads polygynic mating system, with female choice determining the mating success (Wells 2010), might result in both male-biased dispersal or, if males show high territoriality, female-biased dispersal (Cayuela et al. 2020). Sex-biased dispersal in invasive species can both facilitate or limit range expansion by changing the sex ratio and affecting the mate finding mechanisms at the invasion front (Shaw et al. 2018), having consequences similar to an Allee effect (Chuang & Peterson 2016). Similar unbalancing effects on invasion dynamics may be driven by ontogenetic differences in spatial behaviour. Both sex- and size-biased dispersal may thus have important management implications.

Identifying the determinants of spatial behaviour in the invasive toad in Madagascar will allow us to disentangle the effects of spatial sorting from natural ecological processes, while providing important baseline data to inform management strategies.

Toad dispersal can be quantified by measuring the distance travelled between shelter-sites and the frequency of shelter-site change (Shine et al. 2021). As shelter-site selection also represents toads' behavioural response to environmental conditions (Schwarzkopf & Alford 1996), exploring the sheltering behaviour can acquit the twofold purpose of quantifying dispersal rates and identifying dispersal limitations which shape the invasion dynamics in Madagascar.

In this study, we used radiotracking to assess the spatial ecology of invasive Asian toads in Madagascar to (1) test the hypothesis of spatial sorting of dispersal-related behavioural traits and

(2) identify intrinsic and extrinsic determinants of toad spatial behaviour, ultimately obtaining insights into the invasion dynamics.

5.3 Materials and methods

5.3.1 The Asian toad in Madagascar

Duttaphrynus melanostictus is a medium-sized toad (average body size in Madagascar; females = 81.1 mm; males = 72.5 mm; Licata et al. 2021)

. The invasive population reported in the eastern seaport town of Toamasina in Madagascar was likely introduced *via* unintentional transport in commercial containers between 2007 and 2011 (Moore et al. 2015; Licata et al. 2020), and the putative point of introduction was identified in the southwest of Toamasina (Moore et al. 2015) (Fig. 5.1).

The invasion is estimated to spread at a rate of approximately 2 km year^{-1} (FL, unpublished data), expanding in all land-ward directions and habitats (Licata et al. 2019, 2022), which include the urban areas of Toamasina, and the surrounding countryside, characterized by croplands, mixed shrubland, and some remnant patches of degraded forest (Zhang et al. 2020). This region is characterized by a tropical rainforest climate (Peel et al. 2007) with a drier season (August–November; range average rainfall = 85–124 mm/month) and a wet season (December–July; range average rainfall = 181–343 mm/month), with average monthly temperatures ranging from 21°C (July) to 27°C (February) (Merkel 2021). This climate is highly suitable for the *D. melanostictus* lineage that is spreading in Madagascar (Vences et al. 2017). Our study period was characterized by a slightly longer drier season, with the first heavy rainfalls in January (Fig. 5.1).

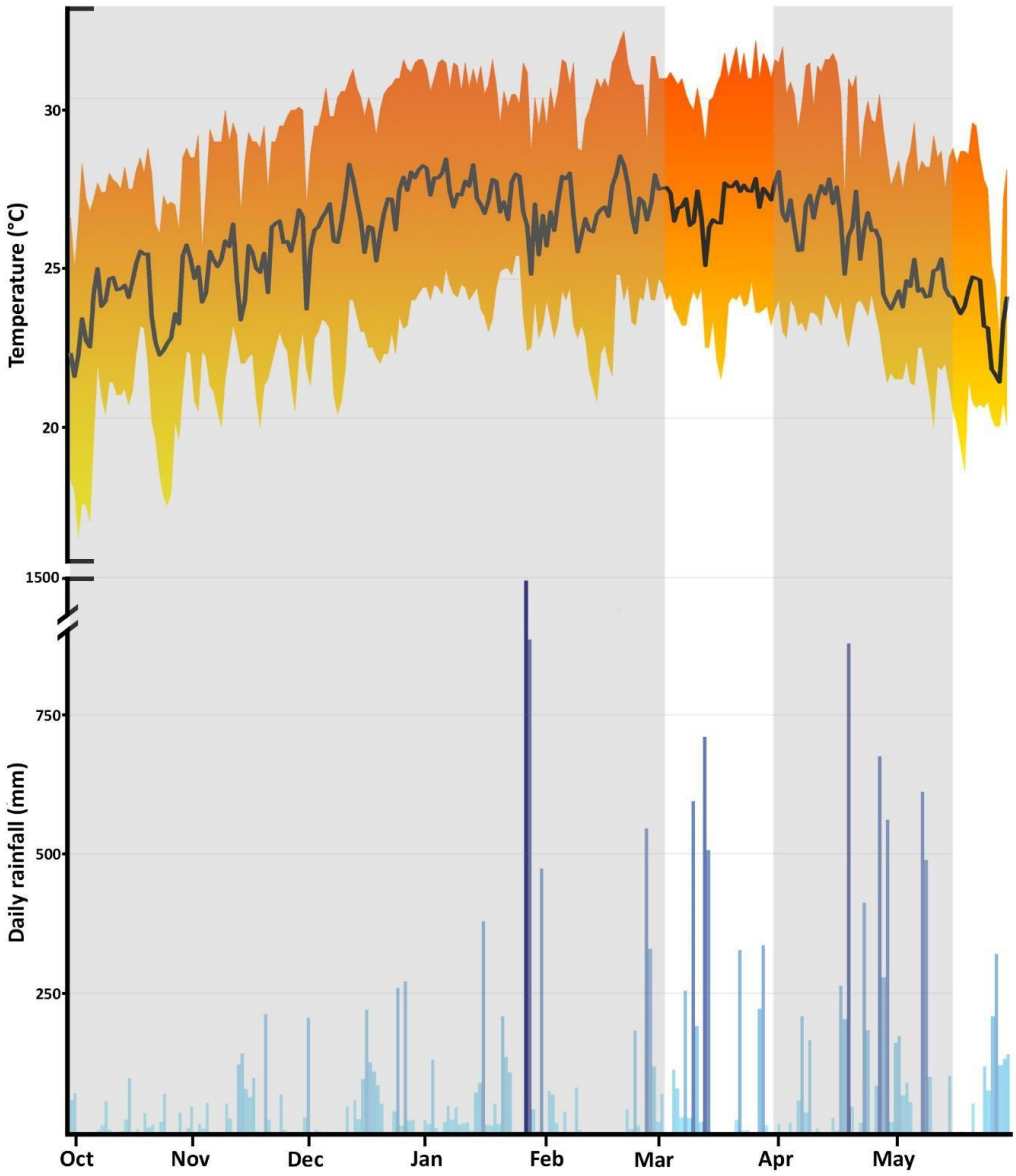

Figure 5.1 - Average daily temperatures and rainfall regime during the study period (October 2018-May 2019). The dark grey areas indicate the periods of radio- tracking of the Asian common toad in Toamasina (Madagascar). Hourly weather data were obtained from the local meteorological station located at Toamasina airport.

The Asian common toad normally breeds in lentic or slow-moving waters, both temporary and permanent, where females can lay up to 10,000 eggs (FL, unpubl. data). The breeding season is largely dependent on the rainfall regime (Jørgensen et al. 1986), and in Central Vietnam, where the climatic conditions are similar to the eastern coast of Madagascar (Ngo & Ngo 2013), occurs in the warmest months with monthly precipitation >100 mm until the beginning of the main rainy season, when the temperatures start to drop (Ngo & Ngo 2013).

5.3.2 Study area

We captured adult toads from three different localities (Fig. 5.2) to study behavioural variation between habitats and between core and front of the invasion.

Ambodisaina (18.148°S, 49.363°E) is a suburban quarter of Toamasina located 5.4 km from the estimated introduction point, where the toad likely established around 2014. This site is in a flat area, near a natural wetland extensively used for paddy fields, and includes several artificial pools used for fish culture and private use.

Vohitrambato Road (from 18.139°S, 49.346°E to 18.103°S, 49.322°E), located 5.6–9.8 km from the estimated introduction point. It is a route which traverses two small villages (<50 houses) in the countryside, interspersed into a landscape characterized by a hilly topography (max. elevation 80 m), rich in wet areas, paddy fields, and streams and dominated by degraded forest and mixed shrubland, with remnant patches of secondary rainforest (Licata et al. 2022). Here, toads likely arrived between 2014 and 2017 (Licata et al. submitted).

Invasion front (18.273°S, 49.254°E), 13 km south of the estimated introduction point, where the invasion front was identified in 2019 (Licata et al. 2021). Toads were found by surveying nearby small settlements along the major road which connects Toamasina to the capital Antananarivo (Route Nationale 2), in a landscape dominated by grassland, mixed shrubland, and paddy fields.

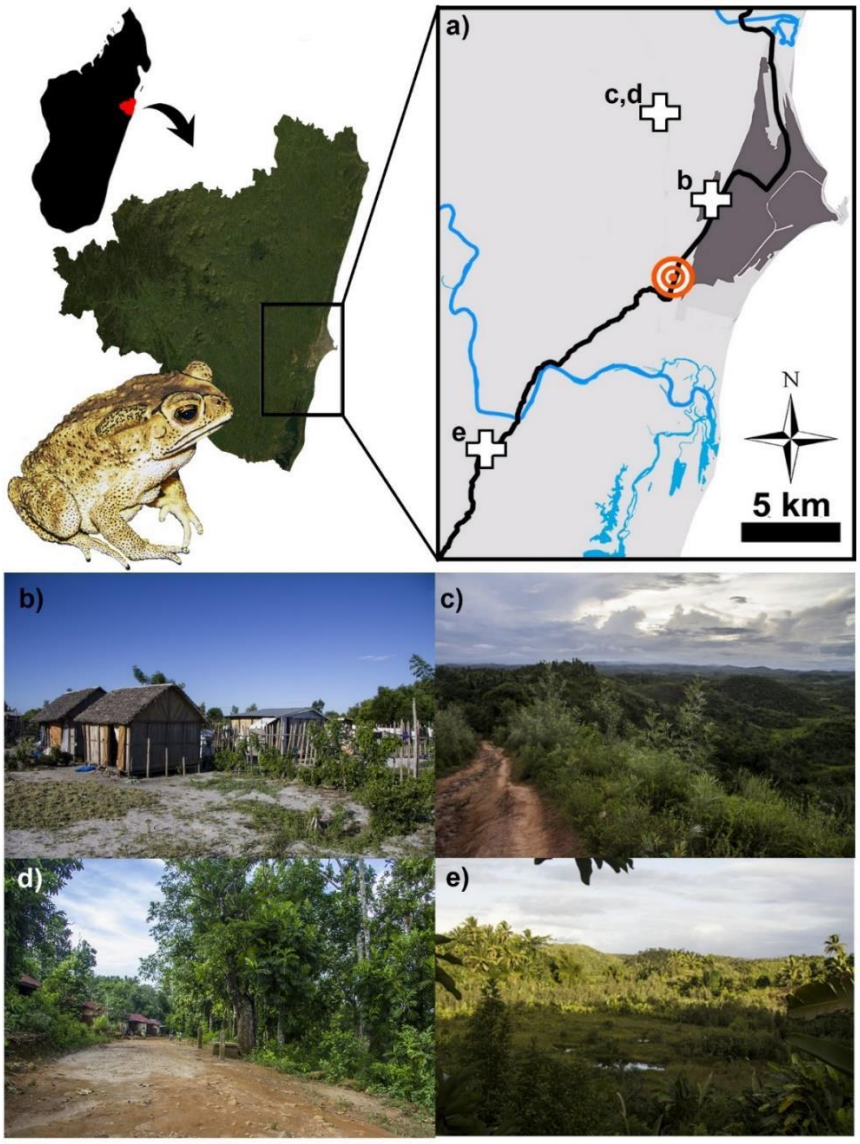

Figure 5.2 - Map of the study area with the sites (white crosses) where the radio-tracking study of the Asian common toad was conducted (a); b) Ambodisaina; c,d) Vohitrambato Road; e) Invasion front.

5.3.3 Radio-tracking

We conducted radio-tracking over 212 days, between the end of the dry season (23 October 2018) and the end of the rainy season (23 May 2019), excluding the period from 4 to 31 March 2019 (Fig. 5.1). After capture, SVL (snout-vent length; mm) and mass (g) of individuals were measured. Sex determination was based on the external characters, with females larger and heavier than males, which in turn have thicker forearms, nuptial pads and develop an orange gular region (Gordon 1933; Licata et al. 2020). Each toad was fitted with a flexible belt around the waist made of silicone rubber capillary tubing (ca. 0.05 g) (Alford & Rowley 2007), holding a radio-transmitter (mass = 2.5 g; model NTF-6-1; Lotek Systems, Ontario, Canada) which weighed on average 7.2% (SD = 2.4) of the toads' mass (Richards et al. 1994) and did not limit their movements. Toads were released back

at their exact capture site within 24 hours. We tracked toads between 11:00 h (approx. solar noon) and 22:00 h (~4h after the sunset). After a toad was located, we recorded the GPS coordinates (Garmin GPSMAP60 CSx; Garmin, Kansas, USA), time of the observation, and shelter type.

We used GPS locations to calculate the displacement between successive shelter-sites. Although breeding activity could have contributed to the spatial behaviour of tracked toads, only movements preceding and succeeding direct observations of breeding toads were considered as migratory movements (e.g., females laying eggs, calling males).

To investigate sheltering behaviour, we determined the type of shelter, distinguishing between natural or anthropogenic, and whether toads changed shelter between consecutive observations. We measured the net displacement as the Euclidean distance between the start and the end point of the tracked toad, and the total displacement as the sum of all movements, while we obtained the path straightness, calculated as the total net displacement divided by the total displacement.

5.3.4 Environmental variables

As weather variables, we considered average temperature (°C), average relative humidity (%), and average precipitation rates (mm) during wet hours (i.e., only considering the rainy hours) between consecutive toad observations. Hourly weather data was obtained from the local meteorological station located at Toamasina airport (5–22 km from the study sites).

As topographical variables, we considered the slope (º) and aspect. Since aspect is a circular variable, the cosine and sine transformations were applied to obtain a continuous variable stressing the north-south and east-west gradients, respectively (northness; −1 south-exposed to 1 north-exposed; eastness: −1 west-exposed to 1 east-exposed) (Lassueur et al. 2006). Topographical variables were derived from ASTER Global Digital Elevation Map (30 m spatial resolution(Abrams et al. 2020)).

As habitat variables, we considered four land cover classes (forest, shrubland, grassland, and barren land), and road density. As toads use water bodies not only for breeding purposes, but also to hydrate (Schwarzkopf & Alford 2002), we calculated the Euclidean distance of shelters to the nearest permanent water body (i.e., natural and artificial ponds/ rice fields) to assess relationship with spatial behaviour. Permanent water bodies were identified from aerial images (obtained from Google Satellite); therefore, we assumed no further water sources were present in the study area.

To obtain land cover, a supervised classification of Sentinel-2 Copernicus satellite imagery was performed using the machine learning approach Random Forests (Breiman 2001) (see Appendix C.1, Table C.4). The distance from permanent waterbodies was estimated using a Euclidean function, while road density was based on a road map obtained from the Geofabrik OpenStreetMap server; www.geofabrik.de).

Before analysis, all data was downscaled to obtaining 20 m spatial resolution grid cells, which maintained enhanced habitat characterization while reducing the computational demand for the modelling procedure. Raster files were manipulated using the R package *raster* (Hijmans et al. 2021).

5.3.5 Statistical analysis

Body condition can influence movement performance of toads (Yagi & Green 2017). Therefore, we calculated the Residual Index (RI), which is considered a reliable body condition index (Labocha et al. 2014) and is obtained by extracting the residuals of the regression of mass against SVL (both ln-transformed). However, also hydration state can affect toad movements (e.g., Vimercati et al. 2018), therefore, we assumed that all toads were similarly hydrated. The frequency distribution of toad movements (i.e., dispersal curve) was fitted with exponential (ln-transformed frequency vs distance) and power-law curves (ln-transformed frequency vs ln-transformed distance).

We used linear mixed-effect models (LMMs) to test the effect of landscape features, weather, and individual characteristics on toad daily movements (i.e., distance travelled between observations obtained on consecutive days), excluding migratory movements. We included sex, body size (SVL) and body condition (RI) as potential predictors of distance travelled. To assess the relationship between distances travelled by toads and weather variables, we included the average humidity, temperature, and the rain in the wet hours between observations. We also included the topographical variables (i.e., slope and aspect) at the starting point of the movement, to assess for geotactic responses in spatial behaviour of toads. As habitat variable, we included the distance from the nearest waterbody at the arrival point of the movement, which is a potential driver of movement in toads (e.g., Tingley & Shine 2011). Finally, toad ID was included as a random effect to take into account interindividual variation.

Prior to building the models, we ln-transformed the response variable, the rainfall, and the distance from water bodies to reduce skewness.

To further assess the role of water proximity on sheltering behaviour, we calculated the mean distance from waterbodies for each toad, which was ln-transformed and used as a response variable in a simple linear regression. For this analysis, we used as explanatory variables sex, SVL, RI of toads, as well as the weather variables between consecutive observations.

We used binomial generalized linear mixed-effect models (GLMMs) to test whether the probability that a toad changed shelter on consecutive days was related to weather and topographic variables, toads' sex, SVL, and RI. As further explanatory variables we included locality, slope, aspect, distance from waterbodies of the shelter previously used (ln-transformed), while toad ID was included as a random effect.

We tested the hypotheses of spatial sorting, sex- and size-biased dispersal on movement patterns of toads, by using path straightness (logit-transformed), net and total displacement (ln-transformed) as response variables. The accuracy of these variables may be strongly affected by interindividual variation of radio-tracking data (e.g., length of tracking period, number of observations per toad). Therefore, we performed weighted least squares regressions, excluding those individuals with less than three observations (i.e., the minimum number of locations needed to estimate path straightness). Weights were given by using the number of observations of toads. As independent variables, we considered the distance from the introduction point, sex, SVL and RI of toads, and the average weather conditions (i.e., temperature, humidity, and rain during wet hours) during the tracking period. We also included the length of the tracking period (ln-transformed), as it can be strongly related to the distance travelled by toads.

To assess the effect of habitat on shelter-site selection, we used a grid-based approach which allowed us to account for habitat availability at the individual level. We created a grid whose center was the cell at which the toad was located before performing the movement (i.e., starting cell), and the rest of the grid represented all cells that could potentially be selected by the toad (i.e., arrival cells). To reduce the computational demand for the modelling procedure, we limited the size of the grid to 49 cells spaced 20 m apart, therefore we excluded all movements above 65 m (0.75 % of movements) which resulted off the grid. We used Generalized Linear Models (GLMs) with binomial error distribution to test two different hypotheses that could explain the probability that a toad moved into a cell. Preliminary analysis using GLMMs showed that toad ID used as random factor did not explain any variation of the dependent variable. First hypothesis (H1): a cell is selected for its habitat characteristics. To test H1, we used land cover values of the arrival cells as explanatory variables (i.e., the proportion of barren land, grassland, scrubland, forest; density of roads and distance from the nearest waterbody). Second hypothesis (H2): a cell is selected for its habitat dissimilarities with respect to the starting cell. To test H2, we used the difference of values between the arrival cells and the starting cell for each land cover variable (e.g., proportion of barren land in the arrival cell minus proportion of barren land in the starting cell). Since the probability of a cell being selected decreased with the distance from the starting point, we included distance of the cell from the starting point and its quadratic term in both models. For this analysis, we selected only daily movements, to avoid uncertainty of cell selection

The correlations between explanatory variables were always <0.7, indicating limited collinearity (Dormann et al. 2013). Prior to running the models, all continuous independent variables were scaled (mean = 0; SD = 1) to allow comparison of estimated parameters.

The modelling procedure was the same for all models: models were built using all combinations of explanatory variables, and ranked on the basis of the Akaike's Information Criterion (AIC) (Burnham & Anderson 2002). AIC corrected for small sample sizes (AIC_c) was used to rank the weighted least-square regressions of path straightness, net, and total displacement,

since we had only one value per toad. We removed all models which had a simpler nested model with lower AIC (or AIC_c) (Richards et al. 2011). For each model we calculated the AIC (or AIC_c) weight, which indicates the relative likelihood of a model given the data and the candidate set of models. For models with binomial error distribution, test statistics were calculated using the likelihood-ratio test. Models were run in R environment (R Core Team 2021) using the packages lme4 for mixed effect models (Bates et al. 2020), lmerTest to obtain test statistics (Kuznetsova et al. 2017) and MuMIn for model selection (Barton 2020).

5.4 Results

5.4.1 Tracked toads

We tracked 44 toads in the suburban quarter of Ambodisaina (24 males and 20 females), 44 toads along the Vohitrambato Route (14 males and 30 females), and three toads at the invasion forefront (1 male and 2 females), where adults were found in low numbers. Four toads did not complete the tracking period either because they died (n=1), lost the harness (n=2) or could not be found (n=1). The tracking period was on average 4.9 ± 2.4 days, with locations obtained on average every 52.1 ± 30.6 hours. Toads from the different localities did not differ significantly in body size (SVL; ANOVA: $F_{2,88} = 0.94$, $P = 0.3$), while a significant effect was found for body condition ($F_{2,88} = 3.31$, $P = 0.04$), with toads from Vohitrambato being slightly leaner than those from Ambodisaina (Tukey's post-hoc test; $P = 0.03$).

5.4.2 Toad movements

The mean distance between shelters travelled by toads within a single night was 15.3 ± 14.6 m (Fig. 5.3). Toads showed a right-skewed, leptokurtic dispersal curve (skewness = 1.6; kurtosis = 5.6), which was best fitted by an inverse power relationship (adjusted $R^2 = 0.58$). Dispersal curves were similar for both sexes (males: skewness = 1.9, kurtosis = 6.3; females, skewness = 1.5, kurtosis = 4.7). During the tracking period, two females moved 88 m and 67 m to reach a spawning site. We considered these as migratory movements, and excluded them from subsequent analyses.

According to the best-AIC model, the distances travelled overnight by toads increased with higher rainfall (LMM; B ± SE = 0.33 ± 0.1; $F_{1,108.1} = 9.35$, $P = 0.002$), and decreased with the distance from the nearest waterbody (B ± SE = -0.46 ± 0.14; $F_{1,101.1} = 9.84$, $P = 0.002$). Furthermore, male toads travelled shorter distances than females (B ± SE = -0.64 ± 0.32; $F_{1,77.4} = 4.05$, $P = 0.04$) (Table C.1).

Total displacement by the end of the tracking period was on average 27.9 ± 31.1 m, with an average daily displacement of 6.3 ± 7.4 m, and was positively related to the length of the tracking period (weighed regression; $F_{1,89} = 10.54$, $P = 0.001$).

The average net displacement was 16.5 ± 19 m, which translates into average net daily displacements of 4.1 ± 5.8 m. Net displacement and path straightness were not significantly related to any variable (Table C.2).

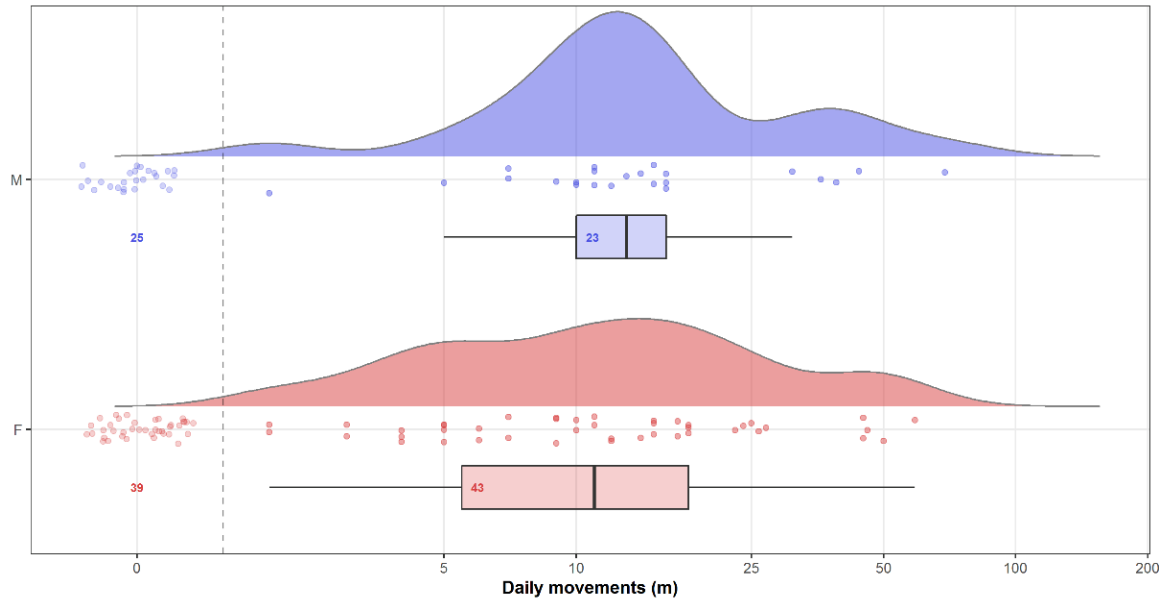

Figure 5.3 - Frequency distribution of daily movements of 91 Asian common toad in Toamasina (Madagascar). Colors correspond to sexes (females = red, males = blue). Box and density plots are plotted excluding non-movements. R code used to obtain this plot was modified from Hodges et al. 2021.

5.4.3 Sheltering behaviour

Toads changed shelter in 54.7% of the observations obtained on successive days, using both natural and anthropogenic shelters, and principally hiding in grass (31.5% of the observations), plant litter (12%), and beneath tree roots (10.2%) (Fig. 5.4a). Toads sought shelter up to 235 m away from waterbodies, with males seeking shelter closer to water than females (B ± SE = -0.99 ± 0.22; $F_{1,88} = 18.6$, $P < 0.001$; Fig. 5.4b, Table C.1). The probability that a toad would change shelter significantly decreased with distance from waterbodies (binomial GLMM; $\chi^2 = 9.05$, df = 1, $P = 0.002$), and increased with humidity ($\chi^2 = 6.04$, df = 1, $P = 0.01$) (Fig. 5.4c). (Table C.1).

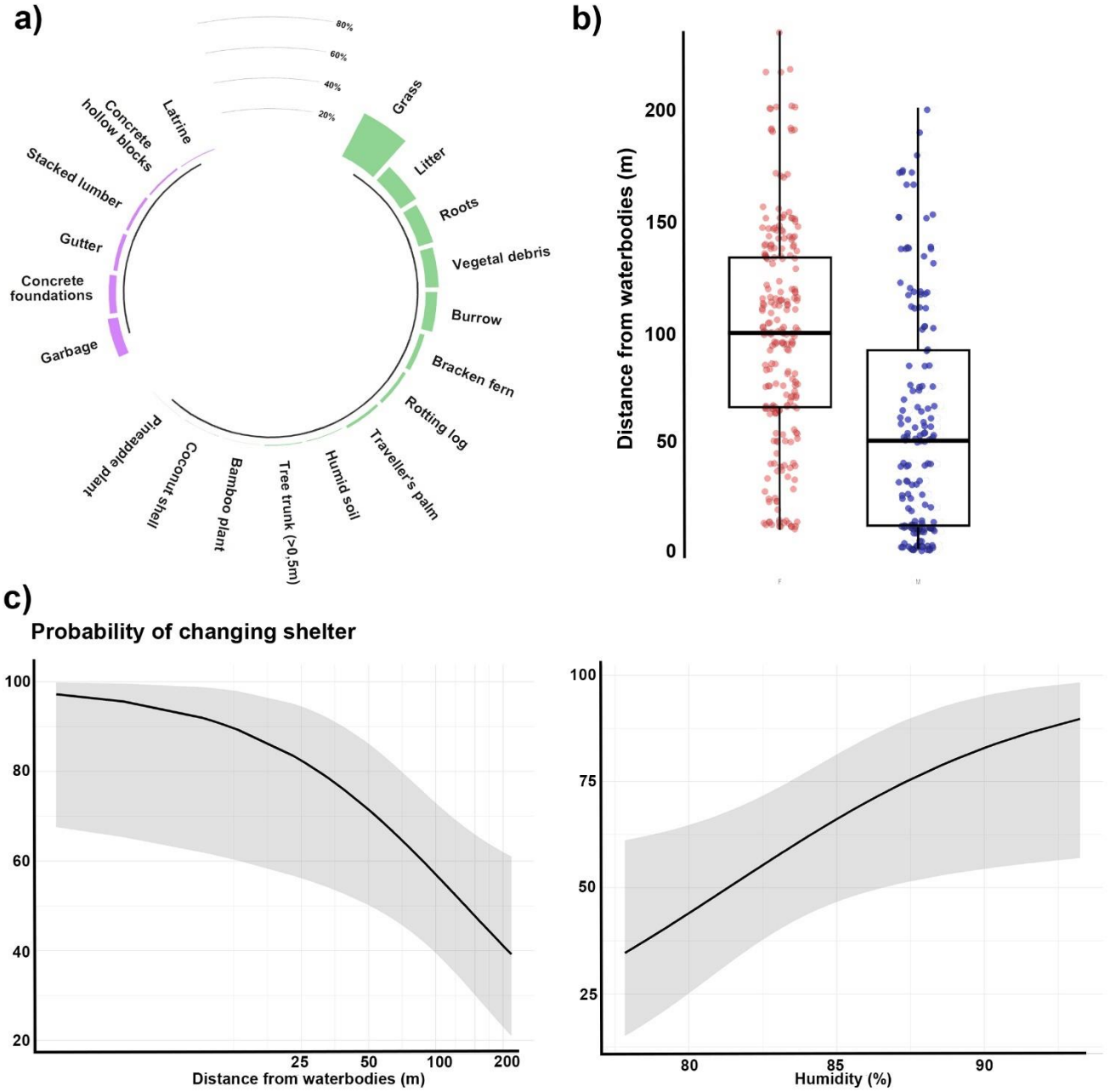

Figure 5.4 - Sheltering behaviour of the Asian common toad in Toamasina (Madagascar). a) Type of shelters (in green natural shelters and in dark grey anthropogenic shelters); b) Boxplot of distances from the nearest water bodies of shelters (in blue males, in red females); c) predicted probability of changing shelter in relation to distance from the nearest permanent water body (ln-transformed; left) and in relation to humidity (right).

Toads inhabited all types of habitats available, and the probability that a cell was selected was not significantly related to any of the land cover variables tested (Table C.3). Conversely, when taking into account the habitat dissimilarity, toads tended to move into areas with higher proportion of barren land (binomial GLMM; $\chi^2 = 3.05$, df = 1, $P = 0.08$).

5.5 Discussion

Tracked toads are habitat generalists showing low displacement rates and a rather philopatric behaviour, but proved able to perform long-distance movements. Dispersal-relevant ethological traits did not change along the invasion gradient, and movements and sheltering behaviour were mostly related to weather conditions and water availability. We found differences in the spatial behaviour between sexes, with males living closer to water bodies than females and changing shelter less frequently.

Altogether, these results support empirical evidence that the range expansion is probably dominated by short-distance leading edge dispersal at this stage of the invasion, however, the long-distance dispersal potential of the tracked toads suggests the possibility of a future increase in invasion speed.

5.5.1 Effects of weather and habitat on spatial behaviour

Toads tended to be more active and moved over longer distances under wetter conditions. Therefore, the end of the wet season (Merkel 2021), is expected to be the most favourable period for toad dispersal. Wet conditions are correlated with evaporative loss rates and are a well-known driver of movement in toads (Muller et al. 2018) and amphibians in general (Linsenmair & Spieler 1998). However, weather conditions had a weak effect on spatial behaviour of toads (Marginal R^2 of highest ranked model = 13%; Table C.1), suggesting that further unaccounted factors, such as food availability, breeding phenology, and reproductive status, may play an important role. Toads changed shelter more frequently and travelled longer distances closer to water bodies (Table C.1), indicating that the availability of freshwater habitat might be a limiting factor for toad movements, at least during the drier season. These findings are consistent with other studies on the spatial ecology of toads (e.g., Tingley & Shine 2011) and could have important management implications. On the one hand, these results suggest that water availability will likely represent an important environmental constraint for toad expansion in the semi-arid south of Madagascar. On the other hand, desiccation risk affects toad propensity to spread throughout the landscape, therefore we expect higher probabilities of toad poisoning effects on native predators around water bodies (e.g., Licata et al. 2022), and broader impacts across the landscape during wet weather conditions (Tingley & Shine 2011).Toad movements did not differ among localities, indicating essential commonality of spatial strategies despite the environmental context. This is also reflected in habitat preferences, with toads found in all types of habitat, although tending to prefer open areas, as is commonly observed in other bufonids (Zug & Zug 1979; Bradford et al. 2003; Brown et al. 2006). Despite no environmental variable was found to significantly affect toad movements and shelter site selection, we observed some individual level variation in habitat use.

5.5.2 Factors determining differences between individuals

Spatial ecology can also be influenced by intrinsic individual factors (Clobert et al. 2009). We found that body condition did not affect movements, and both sexes had similar spatial ecology, although they differed in their spatial distribution. Adult males preferred waterside habitats, as has been documented in other anurans (Wells 2010; Ward-Fear et al. 2016). Asian toads are considered prolonged breeders (Jørgensen et al. 1986), and males can exhibit a fluctuating continuous reproductive activity (Huang et al. 1997), which might induce individuals to stay closer to the breeding sites to maximize the mating probabilities.

Path straightness is a focal metric of spatial ecology often used to determine the dispersiveness of a species and to pinpoint potential trends of adaptive dispersal behaviour, as it represents heritable traits that can undergo rapid evolutionary selection (Phillips et al. 2008; Perkins et al. 2013; Brown et al. 2014). We were not able to detect spatial sorting in this or other behavioural traits along the invasion gradient, which could be due to multiple, non-exclusive reasons, including the short invasion history of this species in Madagascar (Licata et al. 2021). For instance, imperfect dispersal heritability might cause a weak response to spatial selection (Ochocki & Miller 2017) and demographic stochasticity might overwhelm the spatial sorting effect (Kot et al. 1996). However, long-term studies taking into account these processes will allow understanding whether, and to what extent, invasive Asian common toads in Madagascar can undergo spatial sorting of dispersal-relevant traits.

5.5.3 Insights into the spreading dynamics of toads

Madagascan toads' displacement rates (i.e., approx. 4 m per day) stand among the lowest in bufonids (Deguise & Richardson 2009; Mitrovich et al. 2011), and are largely inferior to most of the values documented in both native and invasive cane toad populations (Shine et al. 2021).

Recent estimates indicate that the Asian common toad is spreading at an average rate of 2 km per year following a constant linear spread rate (Licata et al. submitted), which is a pattern often associated with limited species dispersal (Hui & Richardson 2017). This likely explains why *D. melanostictus* is invading Madagascar at a much slower rate than the cane toad at the early stages of the invasion in Australia (10–15 km/year; Urban et al. 2008). Asian toads are smaller than cane toads and have significantly shorter hind limbs, which may result in comparatively lower locomotor performances (Enriquez-Urzelai et al. 2015). Furthermore, the small body mass might limit resistance to evaporative water loss (Junior & Gomes 2015) and thermal tolerance (Klockmann et al. 2017). Further extrinsic, context-dependent factors may have limiting effects on the spread of invasive toads in Madagascar. For instance, conspecific density and breeding success can be important drivers of dispersal, as well as the distance between breeding sites in the landscape (Cayuela et al. 2020). Future studies focusing on these aspects are needed to elucidate the

mechanism underlying the colonization dynamics and rates of emigration or immigration in breeding sites.

The low net displacements of Asian common toads were due to philopatric movements rather than limited activity, and long-distance movements were infrequent (i.e., 2.5% of movements recorded during the dispersal-favourable season), suggesting that range expansion is probably dominated by short-distance leading edge dispersal at this stage of the invasion. However, leptokurtic distribution of toad movements was not exponentially bounded, indicating long-distance dispersal capabilities at the population-level, which, over time, is expected to increase invasion speed (Petrovskii et al. 2011; Lindström et al. 2013). Furthermore, we likely underestimated the long-distance dispersal potential of this species. In fact, we did not consider that five toads were lost (i.e., they potentially moved offsite; see Results), therefore we could have missed their dispersive behaviour (Lindström et al. 2013). Additionally, if long-distance dispersive behaviour occurs infrequently at the individual level, our tracking period may not have been long enough to detect these movements. Despite the large number of tracked toads, we could not assess interindividual variation in space use across seasons or define the home range size of this species. Longer radiotelemetric studies to obtain a full understanding of the ecology of the invasion are needed.

5.6 Conclusion

We have provided the first insights into the spatial ecology of one of the most dreaded invasive species of amphibians worldwide (Measey et al. 2016). Our results have important implications for modelling and management applications, both in Madagascar and elsewhere. For instance, to maximize the outcomes of hand-removal and trapping campaigns in Madagascar, management actions should be focused during the dry season (i.e., August–November), when toads are more sedentary (Tingley et al. 2017), and in open habitats, preferably not in the immediate vicinities of water bodies to target females. Furthermore, since toads sheltered underneath piles of stacked lumber, vegetation debris, garbage, and concrete hollow blocks (Fig. 5.4a), these and similar goods should be prioritized for biosecurity controls, to avert accidental transport of toads to new areas.

The study of biological invasions can be crucial to understand broader ecological and evolutionary processes (Reznick et al. 2019). Baseline data coupled with long-term monitoring of the invasive population will offer the opportunity to explore whether interspecific differences in spatial behaviour may play a role in the spatial evolutionary dynamics of invasive species.

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Chapter 6

Article 5

Toad invasion of Malagasy forests triggers severe mortality of a predatory snake

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6.1 Abstract

The Asian common toad *Duttaphrynus melanostictus* has been introduced to the eastern province of Toamasina in Madagascar, where it is feared to be having devastating effects on native communities by poisoning frog-eating predators. So far it is unclear whether the toad can invade forest habitats, and empirical evidence of its impact on native predators is lacking. We used radio tracking to investigate the spatial behaviour of adult toads in a small parcel of lowland humid forest and we quantify the disruptive effects of toad poisoning on a native frog-eating snake, the Malagasy cat-eyed snake (*Madagascarophis colubrinus*). We used *N*-mixture models to estimate the population size of cat-eyed snakes, and used the mortality events recorded in the same area to obtain an estimate of monthly mortality rate of snakes due to toad poisoning. Our results point to a drastic mortality rate of snakes that, if constant through the year, could halve the predator population, with the potential risk of local extirpation. We expect cat-eyed snake populations across rural and suburban areas of Toamasina to be severely affected by the toad invasion, and suggest that future research should investigate the effects of indirect facilitation of human-commensal rodents and the potential repercussions on human health and well-being.

6.2 Introduction

Madagascar is one of the richest biodiversity hotspots hosting some of the most irreplaceable forests on Earth (Le Saout et al. 2013), where, until recently, invasive species have received only limited attention (Kull et al. 2015). This paradigm is rapidly shifting since the introduction of the Asian common toad (*Duttaphrynus melanostictus*) (VII IUCN World Conservation Congress 2020). Likely having arrived around 2010, the Asian common toad rapidly established in urban and suburban areas of the seaport town of Toamasina (eastern Madagascar) (Crottini et al. 2014; Kolby et al. 2014; Andreone 2014), where it is now relentlessly spreading through rural habitats. Recent estimates report an incursion area of over 500 km^2 and an average spread rate of 2.5 km per year (Licata et al. 2019); complete eradication is therefore highly unlikely (Licata et al. 2020). This toad can reach high abundances in human-dominated habitats, which likely facilitated the establishment of the species in the initial stages of the invasion (Licata et al. 2019, 2020). Invasive toads can affect naïve native communities both by directly affecting specific species or populations, and by disrupting trophic interactions through bottom-up (prey limitation) or top-down (predator removal) effects (Kraus 2015). The invasion of this species in Madagascar represents an unfortunate parallel with the infamous cane toad (*Rhinella marina*) invasion of Australia, which has caused severe declines of several predators, thereby generating trophic cascade effects on Australian ecosystems (Shine 2010). Although the invasion of Asian common toads in Madagascar has received recent attention (Moore et al. 2015; Vences et al. 2017; Marshall et al. 2018; Licata et al. 2019, 2020), studies measuring pathways of ecological and socio-economic impact are lacking (Licata et al. 2020).

The major risk of this invasion lies in the toxicity of the toads, and on the predicted widespread vulnerability of native vertebrate fauna to their toxins, with predator removal due to poisoning being the most feared effect (Marshall et al. 2018). As mesopredators, snakes may play a crucial role in the maintenance of ecosystem functioning, yet are particularly exposed to the effects of the current biodiversity crisis (Zipkin et al. 2020). In Madagascar, several snake species are specialized to feed on lizards and amphibians (Andreone and Luiselli 2000), probably due to the high availability of these taxa in Malagasy rainforests, and may, therefore, be at risk of ingesting the toxic toads. The magnitude of this impact is likely dependent on the ability of toads to colonize rainforests (Marshall et al. 2018), where the abundance of frog-eating predators (e.g., snakes) is the highest (Glaw and Vences 2007). In this study, we explicitly show that the invasive toad is able to colonize densely forested habitats and quantify for the first time the impacts of toad poisoning on an endemic frog-eating snake.

6.3 Materials and methods

6.3.1 Study site

Analabe, located about 6 km west of the seaport town of Toamasina (18°6'18.31"S, 49°19'23.32"E, around 40 m a.s.l.), is one of the few remnant patches of lowland humid forest that survived recent slash-and-burn activities in the region (Fig. 6.1a-b).

This forest fragment covers an area of approximately 17 ha and is characterized by dense but degraded native vegetation with a temporary pond in the central area. This site is included within the Asian common toad invasive range, and was probably reached by the toad between 2014 and 2016 (Licata et al. 2019). Conditions in the region are characterised by a tropical rainforest climate (Peel et al. 2007), alternating between a drier period (August–November) and a wet season (December–July), with monthly temperatures ranging from 21 (July) to 27 °C (February) (Merkel 2021).

Despite being small, Analabe harbors a remarkably rich herpetofaunal community, with at least 18 and 16 species of native Malagasy amphibians and reptiles, respectively. Field investigations were performed from 16 November 2018 to 21 February 2019, and from 23 April to 18 May 2019. We conducted regular surveys in multiple areas of the forest, starting soon after dusk, searching for active toads using headlamps.

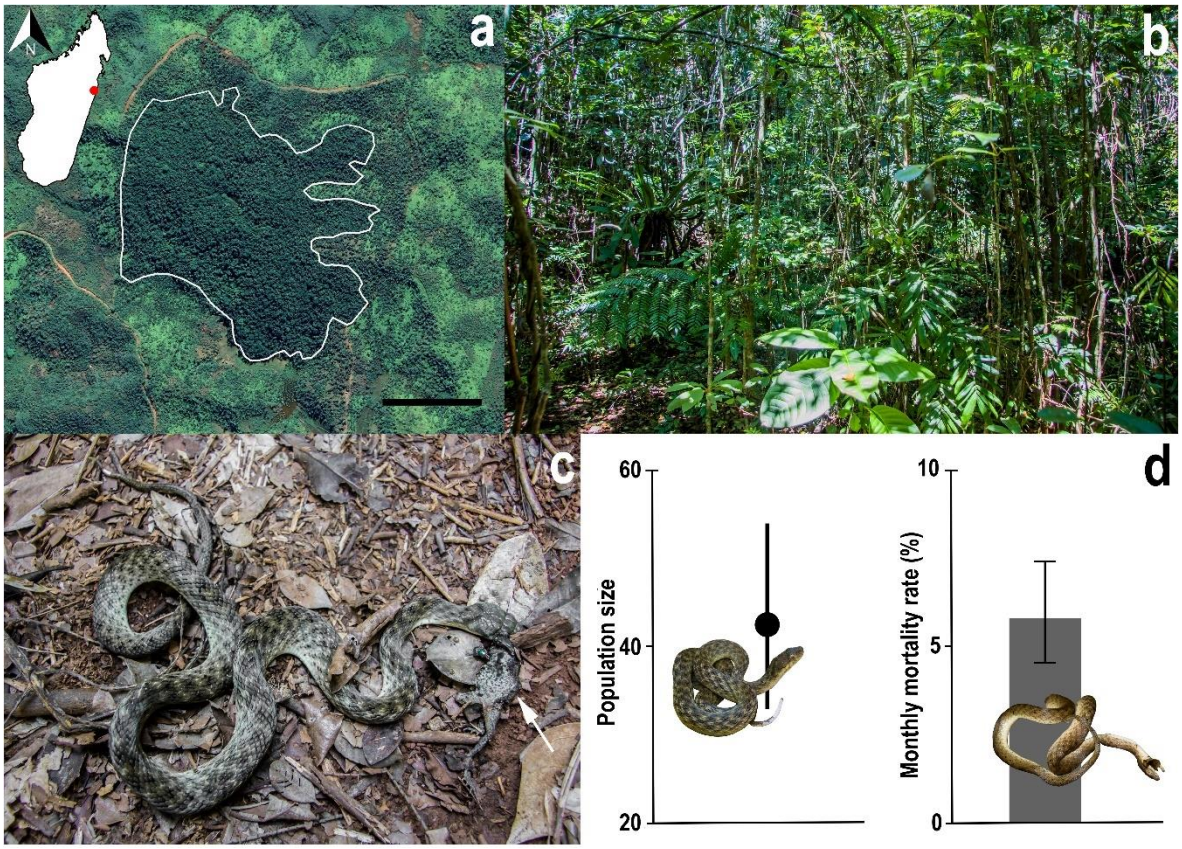

Fig. 6.1 - Toad poisoning effect on *Madagascarophis colubrinus*: a) Analabe forest (scale bar = 200 m) (Map data: Google, Maxar Technologies); b) habitat in Analabe forest; c) carcass of *M. colubrinus* lethally poisoned by a subadult *Duttaphrynus*

melanostictus (arrow); d) estimated average snake population size (left) and monthly mortality rate for toad poisoning in *M. colubrinus* (right), with bars indicating 95% confidence interval.

6.3.2 Radiotelemetry of *Duttaphrynus melanostictus*

Between 23 November 2018 and 12 February 2019, eight adult Asian common toads (*n* = 2 males; mean ± SD; SVL = 74.5 ± 3.5 mm, mass = 30.6 ± 2.8 g; *n* = 6 females, 75.8 ± 3.1 mm, 38.7 ± 5.9 g) were found within or in close proximity to the edges of the forest. We fitted flexible belts around their waists (each belt made of silicone rubber capillary tubing of ca. 0.05 g) (Alford and Rowley 2007) holding a radio-transmitter (mass = 2.5 g; model NTF-6–1; Lotek Systems, Ontario, Canada) which weighed less than 10% of the toads' mass (Richards et al. 1994) and did not limit their movements. Toads were released at the capture site within 24 h.

To investigate the spatial behaviour and microhabitat use of adult toads, we tracked toad positions both during the day and at night, aided by a receiver and hand-held Yagi antenna (White and Garrott 1990). We recorded GPS locations (Garmin GPSMAP60 CSx; Garmin, Kansas, USA), type of shelter and activity status. The tracking period was on average 12.4 days (range = 5–21 days), and toad locations were obtained every 1–7 days (median = 3 days). Using the GPS points, we calculated the average distance between successive locations, the range span (i.e., the distance between the furthest positions recorded), and the Minimum Convex Polygons (100% of locations), to obtain the extent of distribution of locations.

6.3.3 Snake abundance estimates

After detecting the first Malagasy cat-eyed snake (*Madagascarophis colubrinus*; hereafter cat-eyed snake) carcass, we hypothesized that the most likely cause of death was ingestion of toad toxins, and considered cat-eyed snakes to be at risk of mortality where toads are present.

To estimate the abundance of cat-eyed snakes, we surveyed approximately 1,350 m^2 of Analabe. The area was surveyed through regular inspection of five 150 m-long transects in the dense vegetation, and of four plots of approximately 150 m^2 in the area surrounding the pond (hereafter termed sampling units). A preliminary evaluation of the spatial ecology of *M. colubrinus* suggested that the average movement between consecutive observations [distance between consecutive observations = 20 ± 22 m; time between observations = 25 ± 31 days (mean ± SD)] is below the size of our sampling units (F. Licata, unpublished data). We surveyed each sampling unit both during the early (from 29 January to 21 February 2019) and late (from 23 April to 18 May 2019) wet season, to account for variability in activity patterns of the species. Temperature and rainfall regimes changed considerably between the two periods. In April–May, temperatures were on average 2.3 °C lower, while cumulative rainfall was nearly five times higher (4,106 mm vs. 822 mm in the period of January– February). During each period, we visited all sampling units 8

times, for a total of 16 visits over the early and late wet season. Surveys started at dusk, when this species is most active, and two experienced observers counted all active snakes (interval between visits: 3.4 ± 1.9 days; sampling unit visit duration: 10 min).

We used *N*-mixture models, including an extension for open population (Dail and Madsen 2011), to estimate snake abundance from spatially and temporally repeated count data. *N*-mixture models jointly estimate the abundance and detection of animals on the basis of repeat surveys, without the need to capture and mark individuals (Royle 2004). Some studies highlight that obtaining joint estimates of detection probability and abundance can be problematic and *N*-mixture models are sensitive to violations of assumptions (Barker et al. 2018; Link et al. 2018; Duarte et al. 2018). Nonetheless, analyses of real-world data suggested that this approach provides reliable abundance estimates of wild animals (Kéry 2018; Ficetola et al. 2018a) in multiple ecological contexts (e.g., Romano et al. 2017; Costa et al. 2019).

To inform models, we considered three potential detection covariates: time of the survey, temperature at the time of the survey (°C), and total rainfall during the 24 h before the survey (mm). Weather conditions were obtained from the local meteorological station, located at Toamasina airport (* 7 km from the study site). Prior to analysis, detection covariates were scaled to allow comparison of the estimated effect sizes. Models were fitted using a Zero-Inflated Poisson error distribution (ZIP) rather than Poisson models, as the ZIP model showed a lower second order Akaike's information Criterion than the corresponding Poisson model (i.e., AIC_c) (Akaike 1973; Burnham and Anderson 2002). We did not consider Negative Binomial models because of their convergence issues (Kéry 2018). We chose to set the value of the upper limit of integration (K) to ''100 + maximum observed species abundance'', as this value provides stable and robust abundance estimates (Ficetola et al. 2018b). We built models with all possible combinations of detection covariates, and retained the one with the lowest AICc value. The population was not closed between the two survey periods; we therefore tested against the default ''constant'' dynamic scenario, which implies no relationship between apparent survival and recruitment (Dail and Madsen 2011). We tested four possible population dynamics between the two seasons: (a) no temporal trend; (b) exponential trend; (c) Ricker model; (d) Gompertz trend (Hostetler and Chandler 2015), and retained the model with the lowest AIC_c (Burnham and Anderson 2002). We then used empirical Bayes methods to estimate the posterior distribution of snake abundances at the survey sites. We tested the global model for goodness-of-fit by means of a Pearson chi-square test, using a parametric bootstrap procedure (5,000 re-samplings) (MacKenzie and Bailey 2004), which showed that our data were not overdispersed and the model provides adequate fit to the data (*c-hat = 1.18; P = 0.13*). The model building procedure was conducted in R environment (version 3.6.1) (R Core Team 2020), using the package ''unmarked'' for modelling abundances (Fiske and Chandler 2011) and ''AICcmodavg'' to compute the goodness-of-fit test (Mazerolle 2011).

6.3.4 Monthly mortality rates in *Madagascarophis colubrinus*

We used the mortality events observed in the study area to obtain an estimate of monthly mortality rates of snakes due to toad poisoning. To provide a monthly figure of toad poisoning mortality rates we considered a subset (n = 7) of all dead snake observations (Table D.1) collected in the sampling units where snake abundances were estimated between 23 December 2018 and 21 February 2019 and between 23 April to 18 May 2019. We excluded from the analysis of mortality rate those carcasses found outside of the study area where snake abundances were estimated and those for which residence time (i.e., the date of death) could not be established (Table D.1). We averaged the number of dead snakes found over the duration of the survey period (n = 86 days). As decomposing carcasses gave off a strong smell facilitating their detection, and the interval between visits (median = 2 days; range = 1–12 days) was short enough to exclude total decomposition of the carcasses (Parmenter and MacMahon 2009), we infer that we documented most mortality events. Similarly, our observation of snake/toad interactions in Analabe suggest a prompt snake death after biting a toad (see also Results). Nevertheless, it is possible that some dead snakes remained undetected, thus our estimates represent a lower boundary of mortality rates.

6.4 Results

6.4.1 Radiotelemetry of *Duttaphrynus melanostictus*

Toads of both sexes and varying sizes inhabited the forest. The first toad observation within Analabe forest occurred on 23 November 2018, and a total of 18 toads (6 adult females, 2 males and 10 subadults) were observed during the entire survey period.

Adult individuals were found to be resident and showed limited vagility. Eight tagged adult toads were located 26 times (2–7 locations/toad) after the radio tracking device was fitted. Two toads lost their harnesses after 12 and 19 days of radio-tracking, respectively. All movements were recorded within Analabe forest or along its edges. Omitting observations of individuals that remained in the same shelter site on consecutive dates (2 of 26 cases), the mean distance between subsequent positions was 19 m (range = 1.4–55.7 m). The range span of toad locations was on average 28.4 m (range = 3.1–66 m), while the average Minimum Convex Polygon calculated for six individuals was 189.2 m^2 (range = 1–450 m^2). During the daytime, toads sheltered predominantly in leaf litter (16 of 23 cases), often partially or totally exposed, but were also found under rotten tree trunks, between roots, or inside cavities in the ground. In addition to the toads that lost their harnesses, two individuals died after predation during the tracking period. The first individual was found dead on 6 December 2018 after attempted predation by a cat-eyed snake,

which was also found dead in close proximity (Table D.1). The second toad was totally consumed by an unidentified predator (possibly a mammal, based on the marks on the remaining radio-tracking equipment left behind by the predator), and was found on 12 December 2018. The inspection of surrounding areas did not lead to the identification of any carcasses.

6.4.2 Abundances of *Madagascarophis colubrinus*

In our repeated surveys we counted fifty-four cat-eyed snakes (range 0–3 individuals per survey per sampling unit). Snakes were detected in all sampling units. The best AIC_c *N*-mixture model assumed no temporal trend in abundance and suggested that rainfall before the survey positively influenced the detection of snakes ($B = 0.455$, $P < 0.001$; Table 6.1). The estimated per-survey individual detection probability was 0.062 ± 0.058 (mean ± SE) and the estimated total abundance was 41 (95% CI = 31–53) and 44 (95% CI = 36–54) snakes for the first and second survey periods, respectively.

Table 6.1 - Model selection based on Akaike's Information Criterion corrected for small samples (AIC_c), number of parameters (nPars), the difference AIC_c from the best fit models (ΔAIC_c), and model weights (AICcwt).

	nPars	AICc	ΔAICc	AICcwt
A) Initial abundance				
ZIP λ(.)γ(.)ω(.) [Constant] p(.)	5	231.03	0	0.77
Poisson λ(.)γ(.)ω(.) [Constant] p(.)	4	233.47	2.44	0.23
B) Detection				
λ(.)γ(.)ω(.) [Constant] p(Rain)	6	217.15	0	0.99
λ(.)γ(.)ω(.) [Constant] p(.)	5	231.03	13.88	<0.01
C) Dynamics				
λ(.)γ(.)ω(.) [No Trend] p(Rain)	5	215.56	0	0.43
λ(.)γ(.)ω(.) [Exponential] p(Rain)	5	216.92	1.37	0.22
λ(.)γ(.)ω(.) [Constant] p(Rain)	6	217.15	1.59	0.19
λ(.)γ(.)ω(.) [Ricker] p(Rain)	6	218.95	3.39	0.08
λ(.)γ(.)ω(.) [Gompertz] p(Rain)	6	218.95	3.39	0.08

6.4.3 Mortality of *Madagascarophis colubrinus*

Although encountering carcasses of small vertebrates in rainforest habitat is generally a rare event (Rouquet et al. 2005), between 23 December 2018 and 21 February 2019 and between 23 April to 18 May 2019 we found nine cat-eyed snake carcasses in Analabe forest (Fig. 6.1c; Fig. D.1, Table D.1), plus three snake skeletons that could not be identified. All carcasses lacked any evident external injuries or trauma. In four cases, dead toads were found almost intact in the

stomach, mouth, or next to the body of the snakes (Fig. 6.1c; Fig. D.1, Table D.1). In addition, the observation of an adult toad with a clear snakebite mark on the head (Fig. D.1g), suggests that toads can sometimes survive snake predation attempts, despite likely poisoning the snake.

Considering an average population size of 43 (range = 33–54) snakes in the study area, and that we found approximately two poisoned snakes per month in the surveyed area, the resulting average monthly rate of mortality of this population was estimated to be at least 5.7% (range = 4.5–7.4; Fig. 6.1d).

6.5 Discussion

In this study we documented for the first time the successful invasion of the Asian common toad into forested habitats in Madagascar. However, we think that the likelihood and magnitude of the effects of the invasion on rainforest communities will depend mostly on the suitability of forest habitat for toad reproduction, such as the availability of lentic water bodies. The eastern rainforest belt of Madagascar is dominated by the presence of steep slopes and by the occurrence of numerous brooks and rivers (Andreone and Luiselli 2000; Vences et al. 2002). Accordingly, the majority of Malagasy amphibians breed and metamorphose in running waters or phytotelmata (Vences et al. 2002; Glaw and Vences 2007; Strauß et al. 2010). The Asian common toad breeds in lentic waters, which are generally scarce in forested habitat (F. Licata, pers. obs.). Topographic constraints in the forested slopes of eastern Madagascar could limit the spread of the toad invasion, and control strategies targeting suitable breeding sites for toads should be considered to limit the establishment of breeding populations in targeted areas.

The predicted vulnerability of Malagasy vertebrates to toad toxins raises major concerns about the cascading events that this invasion could trigger, although species-specific interactions remain difficult to quantify and predict (Marshall et al. 2018). Documenting the presence of the toad in a forested habitat and quantifying the lethal effects on an endemic snake allowed us to shed light on the possible interactions between an invasive toad and a native Malagasy predator, ultimately bringing into focus the management actions to be undertaken and the major risks associated with this invasion.

6.5.1 Likely interactions with the Asian common toad

Amphibians are an abundant and diverse component of Malagasy rainforests, and several vertebrate species are known to prey on them (e.g., tenrecs, [Olson 2013]; plated lizards [Jovanovic et al. 2009]; freshwater turtles [Luiselli et al. 2011], crocodiles [Wallace and Leslie 2008], carnivores [Farris et al. 2015]). Based on traits likely to affect how these native predators interact with the Asian common toad (e.g., feeding habits, foraging behavior and pattern of activity), nocturnal

carnivores and snakes are the predators that will likely suffer most from the invader (Marshall et al. 2018). Moreover, a tendency for crepuscular activity by the invasive toad also increases the likelihood of interactions with diurnal native predators (F. Licata, pers. obs.).

Madagascar is a hotspot of snake diversity with about 100 endemic species (Uetz et al. 2021), the vast majority of which originated from a single large radiation (Burbrink et al. 2019). Snake communities are mostly represented by small and medium-sized species which exhibit terrestrial or arboreal feeding habits and dietary preferences oriented towards lizards and frogs (Andreone and Luiselli 2000); none of them are predicted to have physiological resistance to toad toxins (Marshall et al. 2018). In addition to cat-eyed snakes, anurophagy has been reported in 11 other genera of Malagasy snakes: *Acrantophis* (Heying 2001), *Alluadina* (Hutter et al. 2018), *Compsophis* (Cadle 1996; Andreone and Luiselli 2000; Eudeline et al. 2015; Hutter et al. 2018), *Dromicodryas* (Andreone and Luiselli 2000; Gandola et al. 2013), *Ithycyphus* (Glaw and Vences 2007), *Leioheterodon* (Rosa et al. 2010), *Liopholidophis* (Glaw et al. 2007), *Mimophis* (Glaw and Vences 2007), *Pseudoxyrhopus* (Andreone and Luiselli 2000), *Sanzinia* (Raxworthy 2003; Glaw and Vences 2007) and *Thamnosophis* (Glaw and Vences 2007; Jovanovic et al. 2009; Eudeline et al. 2015). Yet, dietary preferences of many species are largely unknown. The impact of toad poisoning on each species will depend on multiple factors (e.g., habitat and predator body size; Feit and Letnic 2015) but, among frog-eating snakes, diurnal (e.g., *Dromicodryas*, *Leioheterodon, Mimophis*, *Liopholidophis,* and *Thamnosophis*) or arboreal species (e.g., some *Compsophis* spp. and *Ithycyphus* spp.) are expected to have less interactions with the invasive toad.

6.5.2 Assessing the effects of the invasion

A monthly mortality of 5.7% could potentially have caused a decline of approximately 11% of the population from the first to the second study period. Such a decline was not detected in our second sampling period due to the low detection probability estimated for this population (per-individual detection probability = 0.062 ± 0.05), the short temporal interval between the two sampling periods, and the limited number of sampling sites, as demonstrated in simulation studies (Ficetola et al. 2018b). Despite the apparently territorial nature of *M. colubrinus* (see Material and Methods), a lack of geographic closure of our sampling units between survey periods could have hindered precise estimates of abundance, overestimating monthly mortality rates. A long-term study in multiple invaded/un-invaded localities, with individual identification of snakes (e.g., capture-mark-recapture; Lindberg 2012), will clarify the predator population trends and help obtain a more robust estimate of the toad poisoning effects. If the rate of mortality is similar to our estimates, and it is constant through the year, then annual mortality might be higher than 50% in the studied snake population, a level likely to cause localized extirpation. These figures are nearly double the measured mortality rates of snakes caused by cane toads in Australia (e.g., Phillips et al. 2010).

This difference could be due to multiple context-dependent factors, such as the short time since invasion, implying that no or limited avoidance strategies have been evolved; alternatively, the increased mortality could result from increased activity of cat-eyed snakes during the survey period (i.e., the wet season), which translates into higher probability of interaction with the toxic prey. In addition, habitat suitability for the invasive species (Jolly et al. 2015), and community structure (Radford and Fairman 2015), might also play a key role in determining the impact of toad poisoning (Doody et al. 2009, 2013; Kraus 2015).

Gathering abundance data of target species that exploit habitats at risk of invasion is crucial to assess the long-term effects of toad impact on native communities. However, quantitative information on the abundance of native vertebrates is extremely limited in Madagascar, especially for snakes, which generally have a low detection probability and are difficult to encounter in the field (Durso et al. 2011). The most widespread and easily detectable Malagasy species, which are more likely to suffer from toad poisoning (see above), can serve as ecological indicators of toad impact on native communities, and study of their abundances before and after toad arrival could enable a standardized assessment of the effects of the invasion.

Toad-induced decline of snakes in Madagascar can reduce predation pressure on rodents and insectivores and facilitate their expansion, especially if these species are not impacted by toad poisoning, such as the rat *Rattus norvegicus* (Marshall et al. 2018). An increase of rats may result in reduced harvest yields and stored food quality (e.g., Duplantier and Rakotondravony 1999), and potentially increase the transmission rates of rodent-borne zoonotic diseases (Han et al. 2016). We expect cat-eyed snake populations across rural and suburban areas of Toamasina to be severely affected by the toad invasion. Future research should be directed toward understanding indirect facilitation of human-commensal, disease-bearing rodents after predator removal, and potential relevance to human health. If confirmed, we suggest that the buffering role of snakes should be included under the umbrella of the One Health approach aiming at addressing good health and well-being issues in Madagascar.

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Chapter 7

Article 6

Using public surveys to rapidly profile biological invasions in hard-to-monitor areas

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7.1 Abstract

Understanding the impact and spatiotemporal dynamics of invasive alien species (IAS) in recipient environments is essential for tailoring appropriate management plans. This information can be difficult to obtain in the short term, and intrinsic difficulties of monitoring hard-to-reach areas may hamper prompt estimation of IAS distributions. Using the illustrative case of the invasive Asian common toad (*Duttaphrynus melanostictus*) in Madagascar, we show how public surveys coupled with a multi-analytical approach can promptly provide accurate information on invasion dynamics and impacts. On the basis of key-informant responses, we built polynomial regressions to investigate the spatiotemporal patterns of the invasion, false-positive occupancy models to estimate species occupancy, and mixed-effect models to evaluate the public perception and attitude towards the invasive toad. We found that the invasion is following a linear range expansion of approx. 2 km year $^{-1}$, but man-mediated dispersal is facilitating the long-distance spread of the species. The reliability

of species identification by locals was high; toad occupancy decreased towards the invasion front and increased in the southern portion of the invasive range. Negative public perception was principally dictated by toads' physical appearance and toxicity, decreasing in urban areas, where people were less concerned by toad impacts on ecosystems, and in recently invaded localities, suggesting density- or time-dependent effects. We also identified 12 potential impacts, with "loss of domestic apiaries", "poisoning of poultry" and "decline of snakes" standing out for prevalence and potential severity on local livelihoods and ecosystems. Our results bring important new insights into the invasion dynamics and the complex human-toad interactions in Madagascar, showing the extreme versatility of public survey data to address fundamental requirements for invasion science and management applications, which can be especially useful in hard-to-monitor regions of the world with a low in-country capacity to counter invasive species.

7.2 Introduction

Invasive alien species (IAS) are well-recognized drivers of global and local environmental change, affecting both biodiversity (Bellard et al. 2016) and human well-being and livelihoods (Shackleton et al. 2019b, d). The effects of invasions on ecosystems have long been studied in great detail, while the socioeconomic impacts have only recently been gaining resonance in invasion science, especially since human involvement in biological invasions was acknowledged as part of the problem, and not just as its leading cause (Pejchar and Mooney 2009). IAS may either provide benefits or render local communities more vulnerable to environmental or societal changes, directly altering livelihood systems or facilitating other drivers of disturbance (Vaz et al. 2017); information on socioeconomic dimensions of IAS is thus a prerequisite for setting up management plans (Shackleton et al. 2019a). IAS effects can vary spatially and temporally depending on invasion dynamics, which are in turn regulated by both intrinsic (population and spreading dynamics of the species; (Shackleton et al. 2017; Bradley et al. 2019) and extrinsic factors (connectivity, habitat suitability; (Van Moorter et al. 2021). Taking into account this variability can help optimize resource allocation for invasion management, and mitigate negative societal impacts (Hulme 2006).

Invasion dynamics and impacts represent the minimum suite of information required to characterise a biological invasion, as they provide the basis for quantifying several derived variables and indicators of invasion trends, allowing comparison with other known IAS and the implementation of adequate management measures (Latombe et al. 2017). This information can often be partially available, difficult to obtain in the short term, or may require the implementation of expensive or labour-intensive methods. For instance, invasion dynamics can be traced using computationally expensive agent-based models (Vimercati et al. 2017), which may provide inexact predictions if they do not adequately take into account the context-specific variation and endogenous variability of the study species (Melbourne and Hastings 2009). Similarly, the use of environmental DNA to detect

species presence can be costly, may suffer from detection biases, and requires adhering to high-quality standards to provide reliable information (Ficetola et al. 2015; Smart et al. 2016; Klymus et al. 2020). Traditionally, spatiotemporal patterns of biological invasions are traced using distributional records from herbaria, museums, or grey literature, which can be biased by imperfect detection of species or insufficient monitoring efforts over time and space (Pyšek et al. 2008). Monitoring biases can be particularly exacerbated in countries with limited capacity to act against invasions, which typically lack structured, costly, monitoring plans (Pyšek et al. 2008; Nuñez and Pauchard 2010; Latombe et al. 2017). Furthermore, limited access to remote areas can render difficult the monitoring process, hampering the determination of species occupancy (MacKenzie et al. 2002).

Ideally, invasion monitoring logistics should entail rapid and inexpensive methods, which are easy to implement and can provide the high-quality data required to inform IAS management plans. Involving the public in scientific research may be a valid alternative to more traditional methods, as it facilitates the rapid collection of a large amount of data at broad spatio-temporal scales (Encarnação et al. 2021a) and, at the same time, can improve public engagement. People can collaborate with scientists in planning and implementing scientific projects, or directly contribute with data (Shirk et al. 2012), as is the case with public surveys. The advantages of using this approach lie in its manifold applications. Traditionally, public surveys are used to gather information about people's perceptions, attitudes, and beliefs towards IAS, and their determining factors, which can help to disclose potential socio-economic impacts (Crowley et al. 2017; Ribeiro et al. 2021). Public surveys have been successfully used to estimate the occupancy of IAS, and investigate the determinant factors for their occurrence (e.g., Mohanty et al. 2018; Mohanty & Measey 2019b), but they are also a promising way to reconstruct relatively recent invasion histories, provided that the target species can be easily recognized by the public and the probability of its misidentification is taken into account (Mohanty and Measey 2019).

The characterization of invasion history is pivotal to relate impacts (and their magnitude) to invasion stages (Kulhanek et al. 2011), and to reveal mechanisms of spread (Suarez et al. 2001). A recurrent problem with the use of public survey data to reconstruct invasion histories is the recall error phenomenon (i.e., the decrease of respondents accuracy with time since the event; (Beckett et al. 2001), which limits precise estimate of IAS establishment time in a locality (Mohanty and Measey 2019). Taking into account this phenomenon would greatly improve the reconstruction of invasion histories based on public survey data.

Madagascar is one of the worlds' richest and most threatened biodiversity hotspots (Conservation International 2019). The recent discovery of an invasive population of Asian common toads (*Duttaphrynus melanostictus*) in the eastern coastal province of Toamasina raised fears of an ecological disaster (Kolby et al. 2014) on the scale of the invasion of the cane toad *Rhinella marina* in Australia (Shine 2010), and has the potential to affect people's livelihoods and well-being. In an economically impoverished country such as Madagascar, where even the slightest change in

people's activities can determine the transition to poverty (Thomas and Gaspart 2015), quantifying potential socio-economic impacts of IAS must be considered a priority.

The area affected by the toad invasion is one of the most populated in Madagascar (United Nations 2019), but the topography and poor infrastructure within this area render extensive monitoring efforts quite challenging. The Asian common toad is strongly associated with human-dominated landscapes (van Dijk et al. 2004), and is easily recognizable by locals due to its morphological distinctiveness from native amphibians. These features make it an ideal candidate to test the reliability of public surveys as a tool for rapid monitoring actions and to obtain information on invasion dynamics and potential impacts to be integrated into management strategies.

In this study, we contribute to the application of public surveys in invasion science, emphasizing the use of this method for rapidly profiling biological invasions. First, we evaluate the spatiotemporal dynamics of the invasion and identify the factors shaping the invasion pattern, taking into account recall error in respondents. Second, we evaluate the identification reliability of local inhabitants, by estimating the probability of false-positive detection, and identify determinant environmental factors for the occurrence of the species. Lastly, we evaluate public attitudes towards the Asian common toad, testing variation in responses based on sociodemographic determinants and residence time, and revealing potential socio-economic impacts on the population.

7.3 Methods

7.3.1 The invasive Asian common toad in Madagascar

Duttaphrynus melanostictus is a species complex of medium-sized toads, widely distributed in South and Southeast Asia (Wogan et al. 2016), and often associated with anthropogenic habitats (van Dijk et al. 2004). The Asian common toad invaded several islands of Wallacea (Reilly et al. 2017) and was recently reported in Madagascar, where it was likely introduced via unintentional transport in commercial containers between 2007 and 2011 (Moore et al. 2015; Licata et al. 2020). The putative site of introduction was identified near a nickel and cobalt refinery south of Toamasina (see Fig. 7.1; Moore et al., 2015). In 2017, the invasive toad was reported in an area of 549 km^2 and was expanding in all landward directions colonizing all available habitats, with higher abundances in/around human settlements —especially in presence of garbage dumps (Licata et al. 2019).

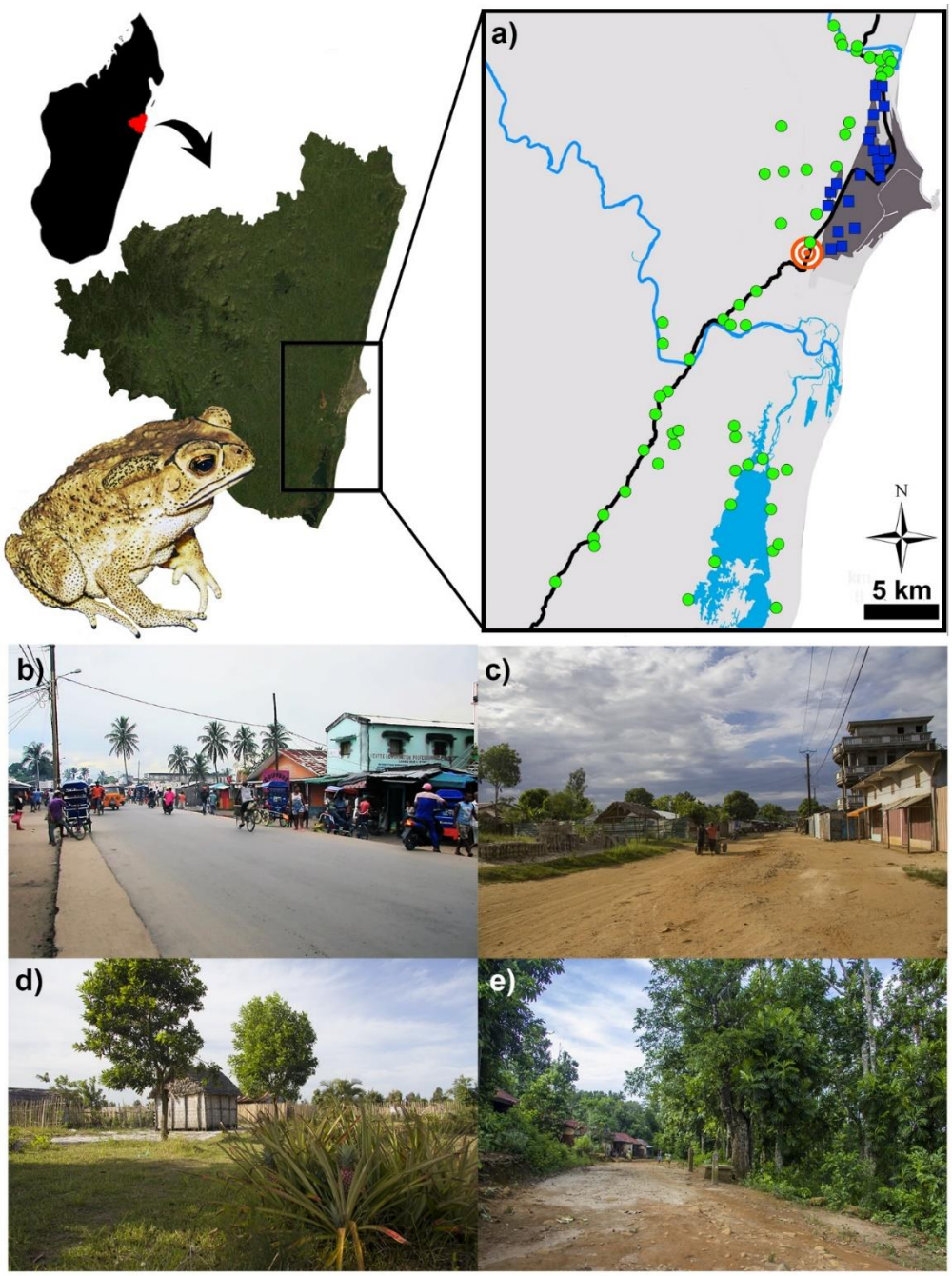

Fig. 7.2 - a) Distribution of the 79 sites surveyed in this study (blue squares = urban communities; green circles = rural communities), with the district of Toamasina I (dark grey area), the putative introduction point (orange bull's eye; Moore et al. 2015), and the road Route Nationale 2 (black line); b, c) urban (along Route Nationale 2) and suburban habitats characteristic of the urban communities; d, e) rural/agricultural and seminatural habitats characteristic of the rural communities.

7.3.2 Study design

We sampled 79 localities from November 2018 to May 2019. Localities were selected in urban and suburban areas of Toamasina (Toamasina I district) and the surrounding countryside (Toamasina II district), and were > 400 m apart (Fig. 7.1a). We categorized localities into two community types. Urban communities were located within the Toamasina I district, and included contiguous suburban peripheral localities (Fig. 7.1b-c), while rural communities included small to

large settlements (>50 households) interspersed in seminatural and agricultural landscapes in the Toamasina II district (Fig. 7.1d-e).

We conducted two different types of surveys: i) visual encounter surveys (VESs) to determine toad occurrence and ii) key informant surveys to reconstruct the spatiotemporal dynamics of the invasion and obtain data on the public perception of the IAS. Two experienced observers conducted VESs starting after dusk (after 6 pm), searching for toads on the streets, green spaces, around settlements, and near water sources. Surveys ended as soon as the presence of the toad was confirmed, or lasted a minimum of 30 minutes and were repeated a second evening to confirm the non-detection of the species (Mohanty and Measey 2019). Overall, we performed field observations in 51 localities (64.5% of the total). Due to security issues, we could not carry out nocturnal VESs at 28 localities (35.4% of the total). As the Asian common toad is strongly synanthropic (van Dijk et al. 2004), we assumed all residents (excluding visitors and new arrivals) have a similar probability of detecting toads, and therefore selected them as key informants. We visited households, courtyards, and local grocery stores to conduct the surveys. Interviews of local inhabitants followed the structure of the questionnaire used by Mohanty & Measey (2019b) and included a combination of structured and semi-structured questions conducted in the local language (Malagasy Betsimisaraka dialect; Appendix E.1). The questionnaire primarily aimed to gather information about invasive toad occurrence ("Have you seen this animal in this particular locality?"), and was supported by a picture of the toad. Upon getting an affirmative response, we elicited information about the time of first observation of the toad in the locality ("When did you first see the toad in this locality?"). Secondarily, we investigated whether respondents perceived a positive or negative impact of the invasive toad ("Do you incur any benefit/loss due to the toad's presence in this village?"; asked as two separate questions), asking to motivate the answer indicating the specific negative or positive effects incurred. Lastly, we asked "Do you kill the toad?", to assess the public attitude towards this species.

7.3.3 Statistical analysis

7.3.3.1 Spatiotemporal dynamics of the invasion

We used people's responses about the time of first observation to estimate the time of the establishment of the invasive toad in each locality, excluding those localities with fewer than three key informants (n=3). For each locality, we calculated the median time of the establishment, along with the median absolute deviance (MAD), which is a robust estimator of relative dispersion that is less influenced by outliers (Arachchige et al. 2020). We used simple linear, second-, and third-order polynomial regressions to investigate the relationship between the median time of establishment and distance from the introduction point. We used weighted least squares to incorporate observational variation into the regression analysis. Weights were given using the reciprocal MAD values,

approximating zero values to 0.5 (i.e., less than one year) to avoid undefined reciprocal values. As independent variables we also considered: i) the effect of the community type, to test whether toads colonized faster urban habitats; ii) the distance from Route Nationale 2 (hereafter RN2), the main road connecting Toamasina to the capital Antananarivo, to assess its potential role as a dispersal corridor; iii) the direction of the locality from the introduction point, to test for differential spread dynamics in opposing directions. The direction was calculated with respect to the coastline, which is offset from the north by 14.6° (Fig. 7.1a). We therefore drew a line through the introduction point which was offset from the north by 14.6°, and used the cosine of the angle between the locality and this line to obtain the direction of the locality (i.e., "1" was equal to 14.6° and "-1" to 194.6°). We ln-transformed the distance from the RN2, to reduce skewness. Furthermore, we scaled all continuous variables (i.e., mean=0; SD =1) for easier comparison of their effects. Collinearity between explanatory variables was low [Variance Inflation Factors (VIF) always < 2], thus we included all of them in the models. We built models using all possible combinations of independent variables and ranked them based on the Akaike's Information Criterion corrected for small sample sizes (AIC_c) (Burnham and Anderson 2002).

We used the predictions of the best AIC_c model to estimate the time of establishment in other localities sampled during previous survey campaigns. To do so, we collated all the available distributional records of the invasive toad published in scientific literature, including one locality (named Analalava) opportunistically surveyed in April 2020 after receiving reports of toad presence (Table E.1), and considered the date of the observation as the minimum time of establishment of the toad in the locality. To identify possible areas of early establishment of toads as compared to model prediction, we retained all localities with the date of observation below a threshold value obtained by subtracting the average MAD value from the predicted time of establishment.

7.3.3.2 Occupancy analysis

We estimated site occupancy using multimethod false-positive occupancy models (Miller et al. 2011). This class of models incorporates detection data obtained from several survey methods characterized by various degrees of certainty, explicitly taking into account the possibility of false-positives, which are intrinsic to some types of dataset (e.g., public surveys, citizen science programs, acoustic surveys) (Pillay et al. 2014; Chambert et al. 2015). This approach allows the use of public survey data as a source of information for species occurrence, considerably reducing the sampling effort as compared to more labour-intensive, time-consuming ecological studies (Miller et al. 2011, 2013; Pillay et al. 2014, 2021). Records from key informants were assumed to be uncertain observations (i.e., both false positive and false negatives are possible), while for our own field observations false negatives were possible but not false positives. We assigned a value of "2" to certain detections (field VESs data), "1" to uncertain detections (public surveys), and "0" to non-

detections. We evaluated the effect of four site-specific covariates in occupancy models: the distance and direction from the introduction point, the community type, and the distance from the RN2. We built models including all potential combinations of independent variables and ranked them on the basis of their AIC_c values (Burnham and Anderson 2002). Variables were transformed and VIFs of all variables were < 2 in this analysis. We estimated occupancy rates (ψ), false-positive detection probability (*p*10), true-positive detection probability (*p*11) and associated 95% confidence intervals. False-positive occupancy models were run using the *occuFP* function in the R package *unmarked* (Fiske and Chandler 2011).

7.3.3.3 Public perception

We treated key informant responses on their perception of the invasive toad as binomial dependent variables. We used generalized linear mixed models (GLMMs) with a binomial error distribution to test the variation in responses as a function of distance from the introduction point and with respect to sociodemographic determinants (i.e., community type, gender, and age of respondents). The distance from the introduction point had a high correlation with the estimated time of establishment obtained from people's response (Pearson's $r = 0.89$; $p < 0.001$), and was used as a proxy of the cohabitation time with the IAS. First, we tested the effect of the selected variables on the primary perceived impact (i.e., "positive", "negative" or "neutral"). Since the questionnaire included semi-structured questions, we obtained specific responses about the perceived impact of toads, which were assigned to four main impact categories (see Results), and likewise considered as binomial dependent variables. We ln-transformed and scaled all continuous variables, to reduce skewness and allow comparison between each variable's estimated effect sizes. In all the models, we considered "locality" as a random effect to account for variation between sampled localities. Also in this analysis low VIF values (VIF < 2) confirmed lack of multicollinearity issues. We built models including all potential combinations of independent variables and ranked them on the basis of their AIC values (Burnham and Anderson 2002). To assess the spatial autocorrelation of the residuals of our model, we performed Moran's *I* test (Legendre 1993). We used the package *lme4* (Bates et al. 2015) to implement GLMMs, *lmerTest* (Kuznetsova et al. 2017) to obtain test statistics, *MuMIn* to build the full set of models (Barton 2020), *ecogenetics* (Roser et al. 2017) for analysis of spatial autocorrelation and *ggeffects* (Lüdecke 2018) to plot model predictions. All analyses were performed in R environment (vers. 4.1.1; (R Core Team 2021).

7.4 Results

7.4.1 Spatiotemporal dynamics of the invasion and identification of outlier localities

Overall, 691 respondents from 43 localities (mean respondents per locality = 15.9; SD = 8.2) provided information about the time of the establishment of the invasive toad. The majority of respondents were from urban communities (57.7%), which were located between 0.98 and 14.57 km from the introduction point. The locality where toads were recorded earliest was 2.76 km from the proposed introduction point, where the median value of responses was mid-2009, while the most recent one (median response = 2019) was located at 14.57 km from the introduction point. The average MAD value was 1.5 years (SD = 1.08; range = 0–4.45), but long-invaded localities showed significantly higher MAD values (Pearson's correlation r = -0.809, df = 41, $p < 0.0001$). The model that best describes the relationship between the time of establishment and distance from the introduction point showed a linear pattern and a very good fit (B = 0.496; SE = 0.036; $p < 0.0001$, R^2 = 0.819), and did not include any other variable (Table E.2). The annual spread rate predicted by the model was 2.014 km year^{-1} (95% CI 1.76–2.36).

Literature revealed several localities where toads arrived much earlier than expected by our model, based on the distance from the introduction point. In 21 out of 154 localities examined the known time of establishment was below the threshold value (i.e., expected time of establishment - average MAD value) (Fig. 7.2). For 16 of these localities, the difference between predicted and observed time of establishment was > two years (Fig. 7.2; Fig. E.1). The most extreme case is represented by the locality named Analalava, located 56 km north of Toamasina, where the toad was recorded in April 2020, 19 years before its expected establishment on the basis of the distance from the introduction point (Fig. 7.2; Fig. E.1).

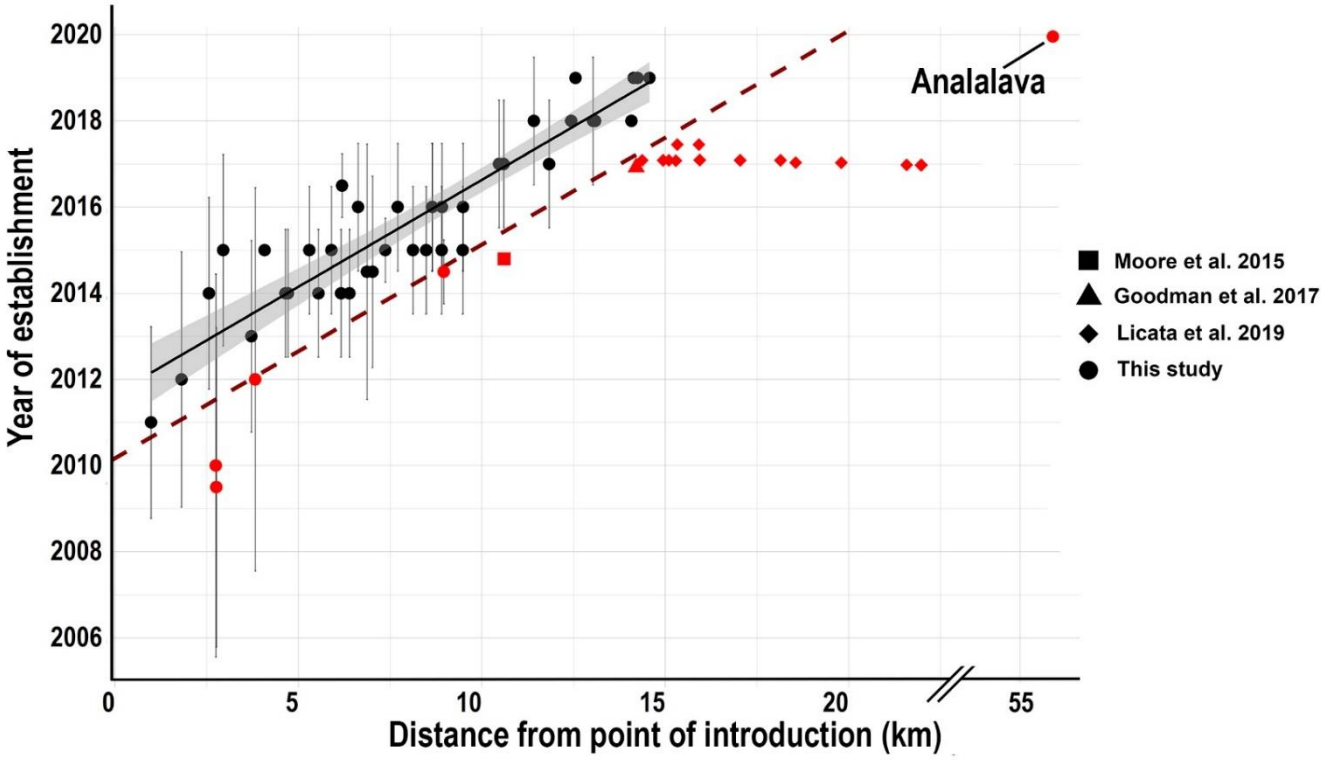

Fig. 7.2 - Result of the weighted linear regression between estimated year of establishment and distance from the introduction point. The estimated year of establishment is obtained from people's responses (median of responses) and the error bars represent the MAD values used for the method of the weighted least squares. In red, the localities lying below the chosen threshold values (i.e., predicted time of establishment – average MAD value) and identified as possible extralimital populations of Asian common toad. Shapes indicate localities published in different articles (see legend).

7.4.2 Detection probabilities and factors determining the occurrence of the Asian common toad

Overall, 1,175 respondents from 79 localities (mean = 14.9; SD = 8.4) provided information on toad occurrence. Field surveys allowed us to detect the Asian common toad in 44 localities located up to 15.5 km from the introduction point, while we did not detect the toad in seven localities located between 13.4 and 17.2 km from the introduction point.

The estimated true positive detection probability by respondents ($p11$) was 0.93 (95% CI 0.91–0.94) while their false positive detection probability ($p10$) was very high (0.01; 95% CI 0.004–0.03). The best model showed a significant negative relationship between toad occupancy and two independent variables: distance from the introduction point and direction, with occupancy rates declining far from the introduction point (B = -5.30; Z = -3.34; $p < 0.001$) and increasing in the southern portion of our study area (B = -0.91; Z = -1.98; p = 0.047) (Fig. E.2, Table E.3, E.4).

7.4.3 Factors determining the public perception of the Asian common toad

Overall, 692 respondents from 49 localities (mean = 14.1; SD = 8.9) answered the attitude survey questions. The average age of respondents was 38.6 years (SD = 14.1 years); 56.5% were women and 57.1% resided in urban communities. Localities were between 0.98 and 14.57 km from the introduction point (mean = 8.3; SD = 3.4).

The 59.5% of respondents reported negative impacts related to the presence of toads, while 38.4% did not report any benefit or loss. The remaining 14 respondents (2%) reported both types of effect (n = 10) or positive effects only (n = 4). The negative perception of the invasive toad decreased significantly as distance from the introduction point increased (binomial GLMMs, Z = -4.59; p < 0.001), while respondents in rural communities had significantly worse perceptions of the toad than did respondents in urban communities (Z = -3.57; p < 0.001; Fig. 7.3a; Table E.5, E.6). Positive responses were nearly zero, as a consequence, factors related to neutral perception showed a pattern exactly opposite to the one of negative perception.

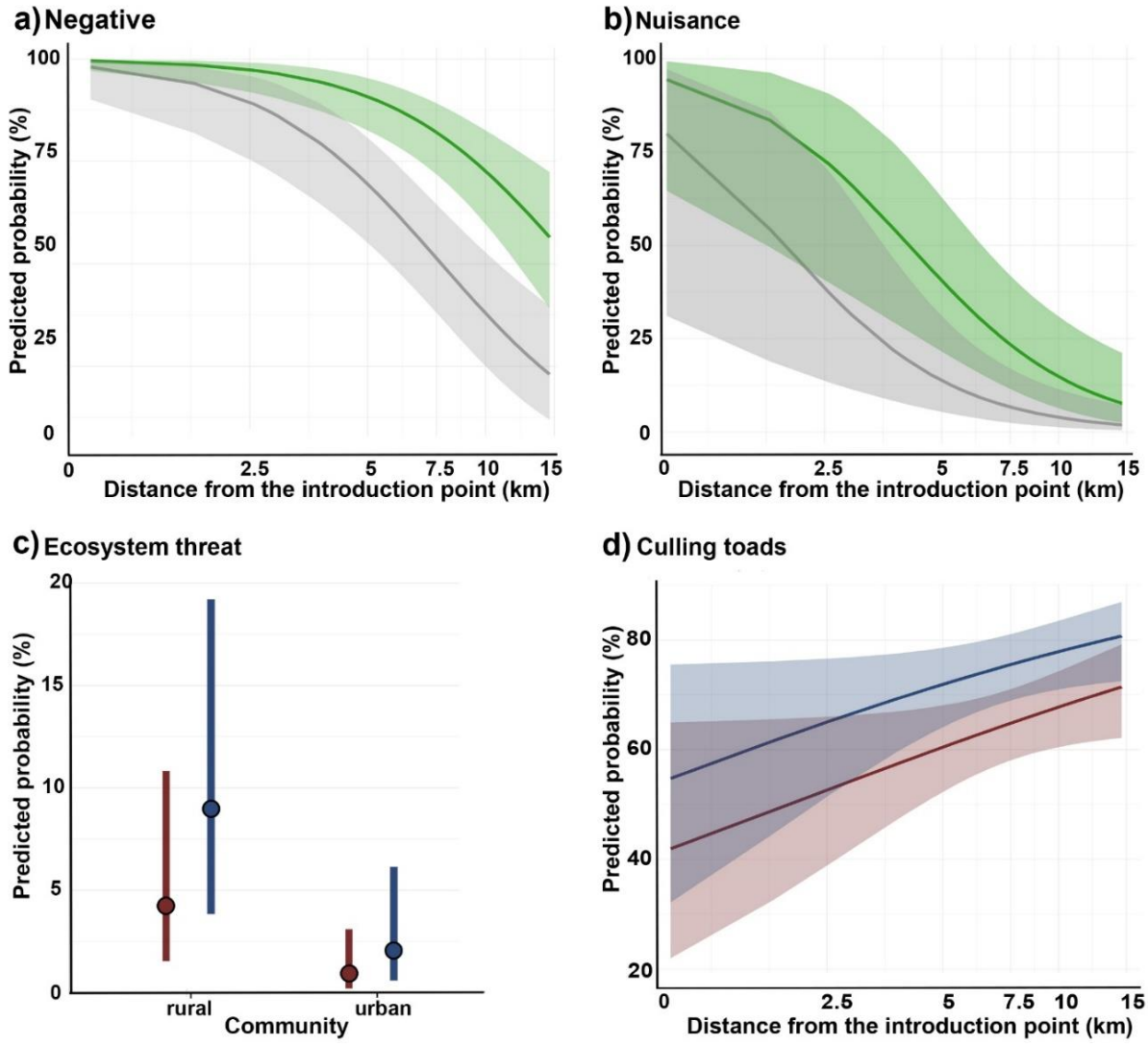

Fig. 7.3 - Predicted probability of response for major (a), and specific (b, c) categories of perceived impacts, and attitude (d) towards the invasive Asian common toad in Madagascar, with the effect of significant predictors, as obtained from public surveys conducted between November 2018 and May 2019. In green, rural communities and in grey, urban communities. In purple, female gender and in blue, male gender. a) Predicted probability of negative response in different community types at different distances from the introduction point; b)

predicted probability of toad's presence being perceived as a nuisance in different community types at different distances from the introduction point; c) predicted probability of toads being perceived as an ecosystem threat between different genders and community types. d) Predicted probability of respondent reporting killing the invasive Asian common toad, as a function of the gender at different distances from the introduction point; Values of distance from the introduction point are ln-transformed.

Responses revealed 12 potential sources of impact by the toad, which we assigned to four main categories: "Nuisance", "Health threat", "Economic threat" and "Ecosystem threat" (Table 7.1). Variation of responses about negative impacts was associated with both sociodemographic determinants and distance from the introduction point. Nuisance was the most reported impact. Toads were considered less of a nuisance in urban communities (Z = -2.21; p = 0.027), and in areas further from the introduction point (Z = -3.63; p < 0.001; Fig. 7.3b; Table E.5, E.6). Toads were also often considered a health threat (e.g., causing skin irritations, polluting water bodies; Table 7.1). The economic threats most frequently mentioned included "loss of apiaries" and "poisoning of poultry", or other livestock/pets (Table 7.1). Health and economic threats were not significantly related to any of the variables tested. The reported impacts on ecosystems included "competition with Indian bullfrog (*Hoplobatrachus tigerinus*)" (which is non-native and invasive in Madagascar but commonly consumed by local communities; (Mohanty et al. 2021) and general concern for environmental degradation, often related to the "decline of snakes" (n = 38), which was sometimes considered as the only positive effect (n = 14). Ecosystem threats were reported more frequently by men (Z = 2.13; p = 0.032) and in rural communities (Z = -2.45; p = 0.014) (Fig. 7.3c; Table E.5, E.6).

Of the 701 respondents interviewed, 482 (68.8%) reported killing the toad. The probability of community members killing toads increased in localities further from the introduction point (Z = 2.01; p = 0.043; Fig. 7.3d; Table E.5, E.6). Overall, men were more likely to kill the toads than women (Z = 2.84; p = 0.004). We did not detect spatial autocorrelation in the residuals of any of the models (Moran's I always < 0.1; p > 0.1).

Table 7.1 - The proportion of respondents reporting a specific impact. Between parentheses the number of respondents and localities from where the specific impact is reported. The total number of respondents in each category may be lower than numbers reported between parentheses as one respondent could report multiple specific impacts.

Impact category	Specific impact	Respondents	Perceived impact
Nuisance		n = 189	
	Toad presence	99.5% (188; 32)	Nuisance caused by the presence of the toad
	Chorus noise	0.5% (1; 1)	Nuisance caused by the chorus noise
Health threat		n = 132	
	Skin irritation	94.7% (125; 29)	Toad's toxicity can induce skin irritation, causing itching
	Poisoning of water	5.3% (7; 4)	Toad tadpoles and adults can poison water sources

Economic threat		n = 77	
	Poisoning of poultry	72.7% (56; 23)	Lethal toad poisoning of poultry
	Loss of apiaries	27.3% (21; 10)	Apiaries loss due to toad predation on bees
	Poisoning of pigs	3.9% (3; 2)	Lethal toad poisoning of pigs
	Poisoning of dogs	2.6% (2; 1)	Lethal toad poisoning of dogs
Ecosystem threat		n = 47	
	Decline of snakes	80.8% (38; 14)	Snakes decline due to toad poisoning
	Competition with Indian bullfrog	10.6% (5; 3)	Indian bull frog declines due to competition with the toad
	Environmental degradation	8.5% (4; 4)	Environment degrades after toad arrival due to toad toxicity
	Increase of rodents	4.2% (2; 2)	Increase of rodents after toad arrival

7.5 Discussion

Biological invasions need to be rapidly profiled in hard-to-monitor, megadiverse regions, to address impacts on ecosystems and human populations. Public surveys can help to rapidly generate essential information to inform possible management interventions, while at the same time improving public awareness. Our combination of multiple analytical approaches allowed us to characterize the range expansion and calculate the invasion speed, ascertain the reliability of occurrence data obtained from the public, and estimate occupancy across the invaded landscape. Finally, the analysis of the public perception data allowed us to describe the complex interactions between invasive toads and humans in different environmental contexts and at different stages of the invasion, and to identify the impacts that cause the greatest societal concerns.

Our work extends prior research (Mohanty et al. 2018; Mohanty and Measey 2019) on the application of public surveys in invasion science by: i) incorporating uncertainty of people responses (recall error) in the reconstruction of invasion history; ii) identifying long-distance dispersal events using deviation from the spread pattern; iii) improving our understanding of invasion trajectories by testing potential drivers; iv) assessing determinants of public perception.

7.5.1 Improving the application of public surveys to investigate invasion dynamics

IAS spread is highly context-dependent, with strongly heterogeneous dispersal patterns (Hui and Richardson 2017). Invasive populations often show a rapid expansion followed by a sigmoidal phase, which reflects population growth dynamics (Hui and Richardson 2017). Some invasions can

also exhibit an accelerating spread because of factors such as spatial sorting of dispersive phenotypes (Phillips et al. 2006) or frequent long-distance dispersal events (i.e., fat-tailed dispersal; (Liu and Kot 2019). Biphasic range expansion can also occur, with two distinct linear spreading rates, which can be driven by a transition to new habitats or stratified dispersal of the species (Shigesada et al. 1995). Lastly, a linear range expansion, with similar distances travelled every year is also possible and is normally associated with a limited dispersal of the species (Hui and Richardson 2017). The use of polynomial regressions to reconstruct invasion histories can help test alternative patterns, whereas including site covariates could inform about determinant factors of establishment success (e.g., community type) or invasion speed (e.g., direction of invasion, distance to the main road), thereby unveiling unexplored hypotheses of spreading dynamics, and ultimately transcend from mere curve fitting. Public survey data have proven reliable in the reconstruction of early-stage invasions, but their application on older invasions was cautioned against due to recall error that hampers fine-scale estimates of establishment time (Mohanty and Measey 2019). Accounting for recall error by introducing measures of statistical dispersion (e.g., MAD) could extend the applicability of this method to older invasions (Mohanty and Measey 2019). This issue could further be addressed by taking into account the level of confidence in respondents' accuracy with specific questions, or identifying respondent characteristics associated with recall error (Beckett et al. 2001), explicitly modelling its determining factors in the study system.

The Asian common toad showed a linear range expansion of approx. 2 km year^{-1} (Fig. 7.2), which is consistent with initial estimates obtained from earlier survey campaigns (1.6–2.5 km year^{-1}; Licata et al. 2019). *Duttaphrynus melanostictus* is currently invading Madagascar at a much slower rate than the cane toad during the early stages of the Australian invasion (approx. 2 vs. 10–15 km year^{-1}; (Urban et al. 2008), which can be due to multiple non-exclusive processes (Appendix E.2). However, other factors are contributing to speed up the invasion. Using deviation from model expectations we detected several outlier localities, where the establishment of the species likely did not follow the normal leading-edge dispersal but could be attributed to long-distance or human-assisted dispersal (Appendix E.3). For instance, the observation of an extralimital, disconnected population 56 km north of Toamasina (i.e., Analalava; Fig. 7.2) confirms that human-aided dispersal is facilitating the invasion, and likely jeopardise the management of this species. Although there is no direct evidence of the dispersal pathway responsible for the introduction in this locality, apart from anecdotal reports of toad arrival with the transport of manure, accidental man-mediated dispersal is likely to occur in Madagascar, due to lack of biosecurity measures for domestic trading (Randriamoria 2019; Licata et al. 2019). Nevertheless, among the outlier localities identified, those closest to the introduction point might suffer from prediction biases caused by recall error (i.e., higher MAD values; see Results), or an offset position of the point of introduction, for which uncertainties exist (Moore et al. 2015).

7.5.2 False-positive occupancy models

The involvement of the general public is pivotal for management actions targeting IAS (Green et al. 2017), but misidentification of target species can pose a serious risk to similar-looking native species, which can be inadvertently killed (Somaweera et al. 2010). False-positive detection rate, although not equivalent, is related to the misidentification rate (Clement et al. 2014), and could provide the first proxy to assess the public's suitability to contribute to IAS management. The distinctiveness and synanthropic nature of the Asian toad probably facilitated its identification by respondents, but greater efforts in selecting key informants may be required in other study systems with habitat specialists, cryptic, or less abundant target species (Mohanty and Measey 2019). Occupancy estimates decreased along the invasion gradient and were higher in southern localities, where invasion could have been facilitated by man-made waterways (Appendix E.2). However, our results only refer to human settlements, which are particularly suitable for the species (Licata et al. 2019), and do not necessarily provide information on natural habitats. Furthermore, our estimates do not consider possible changes in the occurrence status in recently invaded localities, and a dynamic occupancy modelling approach will be needed to evaluate the occupancy dynamics over time.

Occupancy modelling based on public survey data is a well-established method that has found important application in invasion science (Mohanty et al. 2018; Mohanty and Measey 2019). Recent developments in the occupancy modelling framework (i.e., multispecies occupancy models; (Pillay et al. 2021), could improve the use of public survey data for occupancy predictions, allowing to jointly estimate the distribution and determining factors of occurrence of entire IAS communities.

7.5.3 Public perception in the impact assessment framework

Negative perception and attitude towards invasive species are strongly determined by species characteristics (Knight 2008). The toads' toxicity, appearance, and association with unhealthy environments such as latrines may have contributed to the widespread public dislike, however, the reasons raised were largely context- and gender-dependent, and could vary along the invasion gradient. The variation of impacts, and by extension their perception, is the result of multiple factors but is often related to the abundance of the invasive species and to time since establishment (Kulhanek et al. 2011; Bradley et al. 2019). Negative perception and attitude towards the Asian toad followed distinct spatial trends, which were likely dictated either by one or both of these factors (Fig. 7.3a-b,d).

Citizens identified multiple impacts on human well-being, livelihoods, and ecosystems (Table 7.1). On one hand, some perceived impacts (Appendix E.4) could be the result of a shared narrative about the toad's toxicity (maybe originating from public awareness campaigns; see Licata et al.

2020b), which is a phenomenon often reported or inferred for public perception of invasive species (Beckmann et al. 2010; Mohanty and Measey 2019). On the other hand, the consistency of the numerous reports of toad poisoning in poultry, decline of snakes, and loss of apiaries is extremely worrying and deserves further consideration. Poisoning of poultry could represent a major impact on local livelihoods, due to the economic relevance of poultry in Madagascar (Thomas and Gaspart 2015), but actual impacts must be quantified given the contrasting evidence in the literature (e.g., (Beckmann et al. 2010; Gadelha et al. 2014; Marshall et al. 2018). Conversely, many Malagasy native predators are predicted to be vulnerable to toad toxins (Marshall et al. 2018), and at least one species of snake suffers severe mortality rates due to toad poisoning (Licata et al. 2022). The reported decline of snakes in rural and suburban areas might therefore indicate that negative impacts on wildlife are already occurring (Table 7.1). Likewise, the reported loss of apiaries is substantiated by field evidence (Appendix E.4) and may cause substantial problems, especially in recently invaded localities where beekeepers may suffer abrupt losses of beehives. The risks posed by IAS are translated into environmental and socio-economic impact scores (Blackburn et al. 2014; Nentwig et al. 2016; Bacher et al. 2018), which may be subjected to changes when additional evidence of impacts is provided. A previous assessment suggested a 'moderate' socio-economic impact by *D. melanostictus* (Bacher et al. 2018), but the perceived impacts on apiaries and poultry suggest a 'major' impact on human livelihoods in Madagascar.

IAS impacts (both real and perceived) may differ substantially across demographic groups, and weighing up costs and benefits for well-being and livelihoods of different public categories may avoid culturally inadequate or socially unjust management actions (Shackleton et al. 2019d). Rural communities showed a much more negative perception of the toad (Fig. 7.3a-b; Table E.6), which reflected higher perceived impacts (Fig. 7.3c) but could also be related to the effect of the toad's presence on the general landscape aesthetics (Shackleton et al. 2019c). The propensity to kill toads was widespread and univocal between community types, with a decrease of toad culling in long-invaded localities likely driven by the awareness of its inefficacy (Fig. 7.3d), suggesting general public support for this management method. However, this is not always the case, and public acceptance must be evaluated and taken into account to avoid unsuccessful management initiatives (Jarić et al. 2020). Culling by unspecialized people can be problematic, because misidentification errors are possible, with potential impacts on native species (Somaweera et al. 2010). Complicating matters further, the time taken to understand and build community support can make any subsequent management action costlier and less effective (Caplat and Coutts 2011). Public surveys can acquit this twofold purpose by taking the pulse of public acceptance, while at the same time disseminating knowledge and raising public awareness, allowing time optimization over the decision-making process.

Biological invasions will likely increase worldwide in the future (Seebens et al. 2021), but most countries, especially those with a lower per-capita GDP, remain unprepared to counter IAS

spread (Early et al. 2016). New technologies provide a growing panel of tools for species monitoring and detection. Although increasingly affordable (Lahoz-Monfort and Tingley 2018), these resources may not always be easily accessible in regions lacking in-country expertise and structured funding to support long-term management programs. Public surveys can be particularly useful in these circumstances, as they do not require specific technical knowledge, are cost-effective, and extremely versatile. The implementation of this method in Madagascar revealed potential in addressing shortcomings in monitoring capacities, while raw public survey data can be translated into actionable science to inform decision-making, potentially facilitating early intervention against IAS. For invasive species that are easily recognizable and detectable, public surveys are a powerful tool to address fundamental requirements for invasion science. Furthermore, this method may be used in the framework of translational ecology (Enquist et al. 2017), as it creates a direct link between decision-makers, ecologists, and stakeholders. Public surveys can help disseminate awareness, build public support and identify shared strategies between local communities and regulatory agencies, therefore, have the potential to foster integrated management of IAS and improve the probability of successful outcomes.

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Chapter 8

General discussion

Insular biota are extremely vulnerable to biological invasion (Li et al. 2016; Bellard et al. 2017; Fernández-Palacios et al. 2021), especially if they are under the jurisdiction of countries with a limited response capacity to counter IAS (Early et al. 2016). To foster reactive management policies against IAS in these countries, policymakers must be aware of the risk posed to the environment and society, and possess the tools and knowledge to implement IAS management.

Strong taxonomic biases exists in the study of the mechanisms underlying biological invasions (Pyšek et al. 2008), and, among amphibians, most of the studies focus on few iconic invasive species (van Wilgen et al. 2018). Investigating the invasion process of understudied invasive alien amphibians can improve our understanding of the general effects of amphibian invasions on ecosystems and human societies. At the same time, contextualizing idiographic studies in formal frameworks can be relevant to management applications, as it allows generalizations and comparisons with similar case studies (Wilson et al. 2020).

This thesis aimed to shed lights on the mechanisms underlying the invasion dynamics and impacts of the Asian common toad in Madagascar, providing relevant management suggestions and contributing to reduce the bias in invasion science. The management framework of the Asian toad invasion in Madagascar would greatly benefit from building upon other intensively studied model systems; due to toads' toxicity and the lack of native bufonids in both Madagascar and Australia, this toad invasion has clear similarities with the cane toad invasion of Australia (Kolby et al. 2014), therefore the outcomes of this work can be analysed under the 'invasion syndrome' framework (Kueffer et al. 2013; Novoa et al. 2020). The invasion syndrome framework assumes that particular combinations of IAS traits, pathways of introduction, and characteristics of recipient environments may result in predictable invasion dynamics and impacts, which may be addressed using specific policies and management actions, ultimately expediting the risk assessment process and allowing the optimization of management strategies (Novoa et al. 2020).

8.1 Invasion dynamics

The Asian toad increased fivefold its invasive range between 2014 and 2017, which initially suggested an accelerating range expansion (Chapter 3). Morphological and spatial ecology analyses did not detect patterns of spatial sorting of dispersal-relevant traits at the invasion front, suggesting that other factors underlay the observed increase of the rate of spread (Chapter 4, 5). Adult toads showed low net displacement rates, although the frequency distribution of movements was non-

exponentially bounded, thus supporting an invasion scenario initially dominated by short-distance leading edge dispersal with the rate of spread expected to increase over time (Chapter 5). Similarly, analyses of public surveys data confirmed a rather limited range expansion rate of approx. 2 km/year, but highlighted that human-assisted dispersal is contributing to accelerate the invasion (Chapter 7).

The list of alien amphibians include a limited subset of species, which are generally synanthropic and with large native ranges (Tingley et al. 2010). Accidental transport through stowaways on commercial and domestic transport is one of the major pathways of introduction of amphibians (Measey et al. 2017). Both the Asian toad and the cane toad are synanthropic species (González-Bernal et al. 2016; Licata et al. 2019), and are frequently introduced to new regions through human-assisted dispersal (White and Shine 2009; Tingley et al. 2018). In Australia, cane toads are frequently translocated by truck, reaching areas far outside their range of distribution (White and Shine 2009), although establishment events seem to be substantially rare (Macgregor et al. 2021). On the contrary, the Asian toad is likely to have higher establishment rates, considering the short invasion history and the numerous localities identified in Madagascar which were potentially reached through human-assisted dispersal (Fig. E.1; Table E.3). Local agricultural practices might play an important role in facilitating invasion spread in Madagascar, as secondary products of plantations are often used as mulch for other plantations or private use, and since the Asian toad often takes shelter in mulch piles it can accidentally be translocated to new areas (see Chapter 3.5, 7.5.1).

Madagascar's poor road infrastructure may limit the spatial scale of spread through accidental stowaways, whereas the intense national maritime transport passing through the port of Toamasina potentially makes this town a relevant invasion hub (Goodman et al. 2017). Inland waterway transport probably also contributes to the aforementioned dispersal pathways (see Appendix E.3).

Management strategies against the establishment of extralimital populations require early detection through vigilant monitoring and rapid eradication plans (Tingley et al. 2017). Swift eradication actions through hand-removal and trapping of post-metamorphic toads can be highly cost-effective if extralimital populations are promptly detected (Greenlees et al. 2018). To foster an early response, local reports to management authorities can be encouraged through awareness campaigns targeting localities surrounding the main freight corridors and destinations.

In amphibians, invasiveness is often positively related to body size and clutch size (Van Bocxlaer et al. 2010; Measey et al. 2016). As stressed in Chapter 4 and 5, the substantial differences in spread rates between the Asian toad invasion in Madagascar and the cane toad invasion in tropical Australia (Urban et al. 2008) could be partially explained by the fact that cane toads are larger (maximum SVL; 11.8 vs. 23 cm; Reed & Borowsky 1970; Licata et al. 2021), more fecund (average clutch size; 6146 vs. 19472; FL, unpubl. data, Lampo & Medialdea 1996), and have higher displacement rates than Asian toads (Shine et al. 2021) also due to spatial sorting (not observed in the Asian toad; Chapter 4, 5).

Differences in species traits determine population dynamics at the invasion front (Angert et al. 2011). In Chapter 4, I show that adult Asian toads at the invasion front are not larger than individuals at the center of the invaded range, furthermore, adults were few in numbers and outnumbered by juveniles, contrary to what is observed in Australian cane toads (Brown et al. 2013), thus suggesting that both life stages are currently contributing to the invasive range expansion in Madagascar. Natal dispersal is sometimes suggested to represent the highest proportion of total dispersal in amphibian populations (Semlitsch 2008), however, empirical evidence also shows that in some case juvenile-biased dispersal is a byproduct of the fact that juveniles are more abundant than adults (Smith and Green 2006). Invasion-front populations are subject to different selective forces with respect to other population from the range core (Brown et al. 2013). Low numbers of adult toads at the invasion front might be determined by highest mortality rates, before predators' abundances are reduced by toad poisoning (Brown et al. 2013), but also by the low displacement rates of individuals (Chapter 5), which, altogheter, may result in reduced initial reproductive outputs (Brown et al. 2013). If this is the case, juvenile toads observed at the leading edge of the range might originate from populations behind the invasion front, and the rate of range expansion could be influenced by limited initial population growth.

However, unfavorable local environmental conditions may also determine slower rates of spread in different parts of the invasive range (Macgregor et al. 2021). The invasive range of the Asian common toad in Madagascar is climatically suitable for the species (Pearson 2015; Vences et al. 2017). In other bufonids, the high density of available breeding sites has been suggested as a possible limiting factor for species spread (Vimercati et al. 2018). This might also be the case for the Asian toad in Madagascar, where ideal breeding sites (e.g., rice paddies) are very abundant.

If the current rate of spread is maintained, and assuming spatially homogeneous radial expansion, it is likely that in 2022 the invasion will reach the eastern borders of the Ankeniheny-Zahamena corridor, one of the largest areas of rainforest in eastern Madagascar, while it is expected that in the next five years it will reach Betampona Strict Natural Reserve, an isolated patch of lowland rainforest inhabited by numerous microendemic species (Rosa et al. 2012).

Effectiveness of control methods to curtail toad invasions has proven variable and strongly context-dependent, with efforts to be tailored to local situations (Tingley et al. 2017; Greenlees et al. 2020). Fencing of water bodies has proven effective only in arid and semiarid environments (Letnic et al. 2015), therefore its use would be inappropriate in eastern Madagascar, where a large proportion of the terrains is flooded during the wet season. Targeting adult females is the most cost-effective way to reduce toad populations (Reidinger and Miller 2013), and recent studies showed that traps with acoustic lures are more effective than manual removal in Australian cane toads (Muller and Schwarzkopf 2017, 2018). This method could be adapted to Asian toads after testing the efficacy of the acoustic lures, however, being the eradication of toads unfeasible (see Chapter 2.4; McClelland et al. 2015), its use would be limited to mitigating impacts by suppressing toad population size.

Suppression of cane toad populations has limited effectiveness at the invasion front because of continuous immigration (Somaweera and Shine 2012), however, this practice can contribute to limit the co-occurrence with vulnerable native species if it is implemented over long term periods in restricted areas, and it is integrated with other approaches (e.g., prevention of breeding; Tingley et al. 2017).

8.2 Impacts

Asian toads are habitat generalists and can colonize rainforest habitats, where they cause high mortality rates in an endemic predator (the cat-eyed snake *Madagascarophis colubrinus*) through lethal toxic ingestion (Chapter 6). Snakes decline is also expected to be severe in the countryside, where snakes are reported to be disappearing (Chapter 7). Due to its strong synanthropic behaviour, the Asian toad has frequent interactions with local communities, generating a plethora of perceived negative impacts which become stronger with time since colonization. Among these, loss of poultry and domestic beehives stood out for prevalence and potential gravity (Chapter 7).

The naiveté of native predators to the Asian toads, and their physiological vulnerability to toads' toxins, are the characteristic of the recipient environment which mostly determine severe mortality of predators due to toad poisoning. The extent of this effect is related to several intrinsic and extrinsic determinants such as predators' size, dietary preferences, habitat overlap with the invasive species, and ability to develop a rapid behavioural aversion to toads (see also Shine 2010). Toad poisoning effect in Madagascar might be worse than in Australia due to the widespread anurophagy and physiological vulnerability to toad toxins in endemic Malagasy predators (Marshall et al. 2018, Andreone and Luiselli 2000). Important progress has been made to mitigate the effects of toad poisoning (Tingley et al. 2017). For instance, targeted gene flow in vulnerable predators can be implemented if adaptive behaviours are present in populations persisting in toad-invaded areas. This can be achieved by breeding predators coming from long-colonized areas with toad-naïve populations (Kelly and Phillips 2016). Another possibility is using nausea-inducing baits to alter the foraging responses of predators and induce taste aversion to toads (Tingley et al. 2017), a method that, based on species-specific predatory behaviour and dietary preferences, would need to be tailored for each potential predator.

Unfortunately, knowledge gaps on dietary preferences and distribution of Malagasy predators still exist, as well as studies on the ontogenetic variation of toxicity of Asian toad, therefore we are currently unable to compile an exhaustive list of potentially vulnerable predators (e.g., Phillips et al. 2003; Smith & Phillips 2006), or anticipate indirect effects on native communities (e.g., Doody et al. 2015, 2017). Field inventories have been historically focused on legally protected areas (Andreone et al. 2005; Goodman et al. 2018), however, unprotected forest fragments host a high number of microendemic species (e.g., Belluardo et al. 2021). Many understudied and non-protected forest

fragments will be invaded by toads in the immediate future, the risk in these areas is that toad effects on native communities could go undetected, making compensatory conservation measures difficult to implement. Possible effects are not only related to toad poisoning of predators. Invasive toads can directly impact native amphibians through predation, pathogens transmission, and competition (Shine 2014; Falaschi et al. 2020). Available information about the Asian common toad indicates that its diet usually does not include vertebrates (Döring et al. 2017), apart from exceptional cases (e.g., O'Shea et al. 2013). Lethal toxic ingestion of post-metamorphic toads by native amphibians is expected to have a neglectable impact, as anurophagy in amphibians is reported only in a few cases in Madagascar (e.g., Ndriantsoa et al. 2014; Rasolonjatovo et al. 2018). Pathogens transmission to and from native Malagasy amphibians remains largely unstudied. So far, the Asian common toad has not been reported as a potential carrier of important pathogens like the amphibian chytrid fungus, both in its native range (Bai et al. 2012) or in Madagascar (G. M. Rosa, unpublished data) where, nevertheless, *Batrachochytrium dendrobatidis* has been reported (Bletz et al. 2015).

Based on natural history traits, competitive interactions for breeding resources with the Asian common toad can be expected for open-habitat specialists and pond breeders: *Ptychadena mascareniensis*, *Laliostoma labrosum* (although this species is limited to the western portion of Madagascar), *Heterixalus* spp., *Paradoxophyla* spp., *Dyscophus* spp., and some representative each of the genera *Scaphiophryne*, *Aglyptodactylus*, *Boophis* (subgenus *Sahona*), *Mantella*, *Guibemantis*, *Spinomantis*, and *Blommersia*.

Another alien invasive amphibian known to overlap with the Asian toad in the invaded area in Madagascar is the non-native Indian bullfrog, *Hoplobatrachus tigerinus* (Mohanty et al. 2021). The Indian bullfrog is known to prey upon Asian toads of all life stages without suffering lethal intoxication (Datta and Khaledin 2017; Tripathi 2018), and the predatory interaction between these two invasive species may have unpredictable outcomes. Increased predation pressure on toads could help contain the invasion, but at the same time could facilitate bullfrog's spread due to increased availability of feeding resources, or even facilitate both species if density-dependent effects take place (e.g., Abrams 2009). However, different from the Asian toad, no studies have explored the impacts of the Indian bullfrog invasion in Madagascar.

Asian toads may have impacts on local communities' well-being and livelihoods similar or more serious than cane toad invasion in Australia, where the major impact is toad poisoning of totem and bushmeat species important for Aboriginal communities (Bacher et al. 2018). Nowadays, totemism is practically inexistent in Madagascar, therefore toads will have no impacts on local cultural practices, while the decline of relevant bushmeat species vulnerable to toad's toxins (e.g., tenrecs; Jenkins et al. 2011; Marshall et al. 2018) could affect bushmeat consumption in rural areas of Madagascar. Economic impacts such as the loss of farmyard animals and domestic beehives may instead have a disruptive effect on locals' livelihoods, especially in rural areas. In Madagascar, approximately 80% of the population live on less than 1,90$ a day (World Bank 2022), therefore,

even the minimal economic loss could contribute to determine transient poverty (i.e., poverty that can be attributed to intertemporal variability in consumption; Thomas and Gaspart 2015).
Toads may also be natural reservoirs of human pathogens (e.g., *Salmonella* spp., *Leptospira* spp.; Everard et al. 1988; Drake et al. 2013), hence their presence in private gardens and houses might have repercussions on public health.
Similarly, indirect facilitation of human-commensal rodents due to toad-induced decline of snakes might increase the prevalence of zoonotic diseases (Chapter 6.5). In Madagascar, relevant rodent-borne diseases include leptospirosis, hantaviruses (Moseley et al. 2018; Raharinosy et al. 2018), and, most notably, plague, which is endemic to the rural area of the central highlands and is frequently reported in Toamasina (Popova et al. 2018).

8.3 An update of the management framework

We are entering the second decade of the Asian toad invasion of Madagascar and we now have a much clearer picture of the invasiveness, risks, and challenges to face to manage this invasion.
Chapter 2 provides a summary of the first years of activities within the framework of the invasion risk assessment. These were marked by initial difficulties in undertaking concrete management actions, due to multiple reasons. On the one hand, the committee that was created to coordinate the response to the Asian toad, which included Malagasy government representatives, private enterprises, and conservation NGOs, was never officially recognized by Malagasy government, greatly hindering any initial effort to counter the invasion. On the other hand, this committee was excessively inclusive, hampering the decisional process, due to frequent lack of consensus between all involved parties. In addition, it proceeded at a slow pace due to limited funding and the lack of personnel fully dedicated to this cause (i.e., committee's members worked pro bono) (Licata et al. 2020; Freeman et al. 2022) until very recently (2020), when a full time team has been hired to take care of the management of this invasion. Beside these initial difficulties, it is important to stress that most likely, any attempt to eradicate the species upon its discovery (in 2014) was already unrealistic, since by then, the invasive range already occupied an area of at least 108 km^2 (Moore et al. 2015). For comparison, the largest successful amphibian eradication achieved to date was conducted in a much smaller area (0.05 km^2; Wahiawā island, Hawaii, US), which belongs to a country with capacity to counter invasive species already in place (Beachy et al. 2011).
Nonetheless, this daunting situation has not been left unaddressed and instead has led to progress in many respects.
Toad's toxicity and its foreseen impacts on native predators fostered a change of paradigm in the national political agenda of Madagascar, which now includes invasive species. As a result, dedicated posts to tackle invasive species and biosecurity issues have been created within the Ministère de l'Environnement et Développement Durable (MEDD), while managers have been nominated to be

locally responsible for invasive species issues within the regional MEDD teams (DREDD) (Freeman et al. 2022). As a consequence of this, important actions have been taken to tackle the problem of invasive species in Madagascar (e.g., the invasion of the house crow *Corvus splendens*; (Linders and Langrand 2014, CEPF 2022), and most importantly it facilitated the dialogue with IUCN officers, forging important collaborations with local conservation stakeholders. This ultimately led to the acceptance of a motion at the IUCN World Conservation Congress (VII IUCN World Conservation Congress 2020), which, among other things, requested to strengthen existing legislation against IAS in Madagascar, generate a national database of IAS species, and create a national invasive species strategy with the rapid-response capability to promptly remove newly detected IAS (VII IUCN World Conservation Congress 2020). Meanwhile, building national capacity for early detection and rapid response was prioritized through many dedicated workshops for national and regional authorities held in Toamasina, while an emerging class of local scientists and practitioners started developing a greater capacity to deal with invasive species issues in-country (Freeman et al. 2022).

Overall, the slow and cumbersome management framework is finally taking the form of a larger program of control, mitigation, and research led by a heterogeneous group of national and international partners. There is now space for cautious hope that further, significant steps will be taken to cope with the Asian toad invasion (as well other IAS) in Madagascar. However, future benefits of present decisions will largely depend on the timeliness, coordination, and meaningfulness of the measures taken.

8.4 Concluding remarks and future research directions

The invasion of the Asian toad in Madagascar is quite similar to the invasion of cane toads in Australia, due to similarities in species traits, pathways of dispersal, and characteristics of the environments which result in similar invasion dynamics and impacts. Despite interspecific differences exist, these have negligible implications in the management response options available

Impacts may considerably vary between regions depending on national response capability to counter IAS and the inherent vulnerability of recipient environments (Bacher et al. 2018). Cane toad impacts in Australia are largely similar to those hitherto reported in Madagascar. However, national IAS management capabilities and socioeconomic disparity between these countries will likely exacerbate impacts in Madagascar, both on local livelihoods (e.g., Thomas & Gaspart 2015) and native ecosystems (Marshall et al. 2018).

This thesis had a focus on the fundamental biology of the invasive Asian toad population in Madagascar, aiming to elucidate the mechanisms underlying the invasion dynamics and impacts. however, numerous questions still await exploration, and deepening knowledge of the Asian toad biology will enable to fully exploit the management tools used in toad invasions (see Tingley et al. 2017). Following the results obtained in this thesis, I suggest prioritizing the investigation on the

following aspects of research which might help improve our understanding of the invasion and inform future management strategies:

- To improve the interpretability of distributional records it is important that systematic, grid-based surveys recording the covariates that likely affect the detectability of the species are implemented. Furthermore, mapping the invasion front over successive years and in different parts of the invasive range would allow obtaining updated estimates of the spread rate. The methods used in Chapter 7 can effectively serve this purpose, helping to optimize field efforts.

- Habitat suitability models showed that most of Madagascar is climatically suitable for the toad, however, different modelling approaches can help to improve predictions of invasion success. A mechanistic understanding of stage-specific abiotic requirements (e.g., Enriquez-Urzelai et al. 2019) may help to elucidate factors influencing the spread dynamics while, at the same time, inform on abiotic constraints across Asian toad life-stages. Furthermore, using mechanistic microclimate models which take into account vegetation cover and habitat type (Maclean and Klinges 2021), may help to obtain better predictions of future invasion scenarios.

- Understanding the mechanism underlying the colonization dynamics is key to forecasting invasion scenarios. Investigating the breeding sites preferences and the population dynamics of toads in forest and rural areas will be crucial to inform management strategies.

- Field observations suggested that both juveniles and adults contribute to the range expansion (Chapter 4). Investigating the natal dispersal of the Asian toad would improve our understanding of the invasion dynamics of the Asian toad.

- In Chapter 6, I provide a list of species that could serve as ecological indicators of toad impact on native communities. Conducting year-round studies of population dynamics in target species can help to elucidate the temporal variation of the toad poisoning effect, while studying their abundances before and after toad arrival would provide information on the magnitude of impacts on different species, ultimately helping to optimize resource allocation for conservation measures.

- Conducting species inventorying in Madagascar, starting from the invaded areas, is crucial to compile a complete list of potentially vulnerable species. To this end, the dietary preferences of predators should also be studied through molecular approaches (metabarcoding).

- In Chapter 7, I report several potential socioeconomic impacts of toads on local livelihoods. Verifying these anecdotal reports is crucial to set up appropriate management strategies;

Implementing a multidisciplinary approach which integrates socioeconomic and biological data would allow to understand the impact dynamics across different community types, while quantifying and relating the magnitude of the impacts to time since colonization and/or toad abundances will help elucidate the complex human-toad relationship in Madagascar.

8.5 References

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Appendix A

Chapter 3

Article 2

Abundance, distribution and spread of the invasive Asian toad *Duttaphrynus melanostictus* in eastern Madagascar

Table A.1 - List of localities surveyed indicating presence (1) or non-detection (0) of the Asian toad

Date	Site	latitude	longitude	presence/non-detection
02/11/2016	Tranomora	-18.15055	49.37145	1
03/11/2016	Brickaville	-18.81996	49.06826	0
04/11/2016	Vohitrambatoeglise	-18.1221	49.33485	1
07/11/2016	Vohibolo	-18.13883	49.29419	0
07/11/2016	Sahavilarna	-18.13866	49.30616	1
16/11/2016	Analamalotra	-18.07819	49.39103	0
17/11/2016	Mangarano	-18.14296	49.37916	1
22/11/2016	Barikadimy	-18.12826	49.38067	1
25/11/2016	Farafaty	-18.14259	49.35754	1
29/11/2016	Antanimarina	-18.12712	49.36129	1
30/11/2016	Mahasoa	-18.23097	49.30517	1
08/12/2016	Ambodibonara	-18.13691	49.34990	1
09/12/2016	Amparihilava	-18.16865	49.36865	1
13/12/2016	Ambalamanasy, carr3	-18.10336	49.38776	0
20/12/2016	Ambalamanasy	-18.11085	49.38601	0
21/12/2016	Hameau 11 B	-18.11206	49.32826	1
21/12/2016	Ranomena	-18.10705	49.32126	0
21/12/2016	Ampiranambo	-18.06342	49.18343	0
21/12/2016	Ambodimangabe	-18.06105	49.18599	1
22/12/2016	Antanamakoa	-18.08189	49.17393	1
22/12/2016	Ambodizarina	-18.13875	49.29425	1
22/12/2016	Ambatofotsy	-18.08038	49.17038	1
22/12/2016	Ambodilazana	-18.14083	49.31757	1
22/12/2016	Ambodisaina	-18.13951	49.32941	1
12/01/2017	Fanandrana	-18.25356	49.26787	1
12/01/2017	Sahave	-18.23001	49.28287	0
12/01/2017	Andohan'Ivango	-18.15128	49.16043	0
12/01/2017	Ambalanaomby	-18.21338	49.28804	1
12/01/2017	Vohilavacarriere	-18.20841	49.28598	1
12/01/2017	Ampangadiampasika	-18.15128	49.16043	1
12/01/2017	Mizimbola	-18.15128	49.16043	1
12/01/2017	Antananambo	-18.15482	49.17166	1
12/01/2017	Antetezambaro	-18.00169	49.24229	0
12/01/2017	Analamalotra	-18.04027	49.22421	0
13/01/2017	Ambodibonara EPP	-18.18311	49.14163	0
13/01/2017	AntanmbaoAmbodibonara	-18.33237	49.22457	0
19/01/2017	Andranoampango	-17.41244	49.29474	0
19/01/2017	Analalava	-17.48157	49.30406	0
19/01/2017	EPP	-17.40397	49.30406	0
19/01/2017	DoboMahavelona	-17.67642	49.51542	0
19/01/2017	Antaratasy	-17.76562	49.48426	0
19/01/2017	Ambohimarigny	-17.80437	49.47845	0
19/01/2017	Antanambao Nosy be	-18.19565	49.13284	0
19/01/2017	Ambodiatafana	-17.95285	49.43621	0

20/01/2017	PisteAnjinjanaomby	-18.18676	49.29616	1
25/01/2017	Ambodimandresy	-18.04000	49.21400	0
25/01/2017	Ambodiatafana	-18.04050	49.21400	0
25/01/2017	Sahabefoza	-18.04105	49.21405	0
29/01/2017	Analabemazava	-18.05477	49.31960	1
01/02/2017	Tapakala	-18.22797	49.36343	1
01/02/2017	Amboditandroho	-18.16230	49.20337	1
01/02/2017	AndranompanihyManitra	-18.28532	49.33670	1
01/02/2017	Ampasindava	-18.19063	49.18179	1
01/02/2017	Vohiteza	-18.26175	49.32847	1
01/02/2017	Ambodisiny	-18.15515	49.21234	1
01/02/2017	Tapakala	-18.13407	49.21483	1
01/02/2017	Sahafosa	-18.17349	49.17239	1
01/02/2017	Vohimarina	-18.30600	49.31944	1
02/02/2017	Ambodiatafana	-18.32624	49.29393	0
02/02/2017	Ampasindava	-18.18244	49.15246	0
02/02/2017	Ambatonalagnona	-18.17325	49.16427	0
03/02/2017	Bac Ampasindava	-18.31991	49.31065	0
03/02/2017	Ampiranambo	-18.18207	49.15191	0
03/02/2017	Ambohitranivotra	-18.30647	49.25448	0
03/02/2017	Marotanindrazana	-18.30584	49.25178	1
03/02/2017	Bac Ambodibonara	-18.31381	49.29570	0
03/02/2017	PisteAmbatovy	-18.30678	49.25683	0
03/02/2017	Antanandava	-18.31056	49.24024	0
15/02/2017	Tanambao V parcelle 12/13	-18.14543	49.40668	0
15/02/2017	Tanambao V parcelle 12/12	-18.14353	49.40464	0
15/02/2017	pontBefroaka	-18.14448	49.40155	0
15/02/2017	Tanambao V parcelle 13/81	-18.14460	49.39890	0
15/02/2017	Ankirihiryparcelle 11/21	-18.14550	49.39529	1
15/02/2017	Ankirihiryparcelle 11/14	-18.14619	49.39471	0
15/02/2017	La Poudrette	-18.15017	49.39403	0
15/02/2017	Tanambao V parcelle 11/43	-18.14959	49.39608	0
15/02/2017	Tanambao V parcelle 13/71	-18.14700	49.39814	0
15/02/2017	Tanambao V parcelle 13/72	-18.14569	49.39935	0
15/02/2017	Tanambao V parcelle 13/45-46	-18.15060	49.39501	0
16/02/2017	Moraranoparcelle 21/15	-18.16153	49.39515	0
16/02/2017	Pont verrerie	-18.16251	49.39363	1
16/02/2017	Morarano parcelle 21/62-63	-18.15623	49.39114	1
16/02/2017	Morarano parcelle 21/61	-18.15558	49.39036	1
16/02/2017	Pont ambalakisoa	-18.15575	49.38993	1
16/02/2017	Moraranoparcelle 21/43	-18.15298	49.39144	1
16/02/2017	Moraranoparcelle 21/42	-18.15264	49.39230	0
16/02/2017	Poste Ambolomadinika	-18.15113	49.39331	0
16/02/2017	Pont Ambolomadinika	-18.15085	49.39286	1
16/02/2017	Vazahagasy	-18.15176	49.39402	0
17/02/2017	DREEF Atsinanana	-18.15581	49.40153	0
17/02/2017	Depot parcelle 23/44	-18.17445	49.39528	1
17/02/2017	Depot Deuxieme	-18.16756	49.40327	0
17/02/2017	Depot port fluviale	-18.16318	49.40182	0
17/02/2017	Androranga parcelle 21/12	-18.16259	49.40027	0

17/02/2017	Androranga parcelle 21/23	-18.16224	49.39944	0
17/02/2017	Tanamborizano parcelle 22/22	-18.16252	49.39910	0
17/02/2017	Tanamborizano SKT	-18.16438	49.39663	0
17/02/2017	Tanamborizano parcelle 22/22-23	-18.16488	49.39598	1
17/02/2017	Tanamborizano parcelle 22/31	-18.16521	49.39559	1
17/02/2017	Tanamborizano Canal	-18.16679	49.39446	1
17/02/2017	Tanamborizano parcelle 22/32	-18.16662	49.39437	1
15/06/2017	Vohidrotra 1	-18.06078	49.40644	1
15/06/2017	Vohidrotra 2	-18.06175	49.39456	1
30/09/2017	Secrimad	-18.12619	49.36214	1

Appendix B

Chapter 4

Article 3

Does spatial sorting occur in the invasive Asian toad in Madagascar? Insights into the invasion unveiled by morphological analyses

Table B.1 - Morphological traits of the Asian common toads of Madagascar divided by sex (mean ± SD). SVL, snout-vent length; HW, head width; RUL, radioulnar length; TBL, tibiofibular length.

Traits	**Male**	**Female**
	(n = 89)	**(n = 139)**
SVL (mm)	72.48 ± 6.80	81.1 ± 11.4
Mass (g)	34.20 ± 12.13 (n=87)	49.09 ± 23.49 (n=136)
HW (mm)	26.12 ± 2.27	29.31 ± 3.67
RUL (mm)	22.22 ± 1.83 (n=73)	23.38 ± 2.62 (n=112)
TBL (mm)	28.00 ± 2.70	29.46 ± 3.74
HW ratio (%)	36.08 ± 1.34	36.24 ± 1.82
RUL ratio (%)	30.96 ± 1.28	29.26 ± 1.38
TBL ratio (%)	38.65 ± 1.40	36.50 ± 1.62

Table B.2 - List of polynomial models computed to assess allomorphic relations between morphological traits (HW, TBL and RUL) and SVL, including the effect of sex. The best models are in bold. SVL, snout-vent length; HW, head width; RUL, radioulnar length; TBL, tibiofibular length.

Trait	Formula	d.f.	AIC	R2 Adjusted	ΔAIC
HW	**Sex + SVL^3**	**1,223**	**-815.1**	**0.892**	**0**
	Sex + SVL^2	1,224	-814	0.891	1.1
	Sex + SVL	1,225	-813.6	0.89	1.5
	Sex + SVL^4	1,222	-813.1	0.891	2
RUL	**Sex + SVL^3**	**1,180**	**-683.3**	**0.864**	**0**
	Sex + SVL^2	1,181	-682.9	0.863	0.4
	Sex + SVL^4	1,179	-681.6	0.863	1.7
	Sex + SVL	1,182	-680.1	0.86	3.2
TBL	**Sex + SVL^2**	**1,223**	**-836**	**0.893**	**0**
	Sex + SVL^3	1,222	-834.4	0.893	1.6
	Sex + SVL	1,224	-833.8	0.892	2.2
	Sex + SVL^4	1,221	-832.4	0.892	3.6

Appendix C

Chapter 5

Article 4

Insights into invasion dynamics of the Asian common toad in Madagascar from spatial behavioural analyses

Table C.1 - List of best models (LMMs, LMs, and GLMMs, respectively) relating movements and sheltering behaviour of the Asian common toad in Toamasina (Madagascar). Number of parameters estimated in the model (K), AIC score of the model, AIC difference with the best model (ΔAIC), the relative weight of the model with respect to the candidate set of models (wAIC), variation explained by the fixed effects (R^2M = marginal R^2) or by both fixed and random effects of the model (R^2C = conditional R^2), variation explained by the models adjusted for the number of parameters (Adj R^2). Only models with 4<ΔAIC and null models are shown. In the best model, significant variables are highlighted in bold.

Response **Model formula**	**K**	**AIC**	**ΔAIC**	**wAIC**	**R^2M**	**R^2C**	**Adj R^2**
Distance between shelters							
Distance water + Rain + Sex	6	438.99	0.00	0.40	0.13	0.41	-
Distance water + Rain	5	440.52	1.52	0.19	0.10	0.36	-
Distance water + Sex + Humidity	6	441.89	2.90	0.09	0.11	0.41	-
Rain	4	441.98	2.98	0.09	0.05	0.31	-
Distance water + Humidity	5	442.89	3.90	0.06	0.09	0.36	-
[…]							
Null	3	444.77	5.78	0.02	0.00	0.26	-
Distance of shelters from waterbodies							
Sex + Temperature	4	275.67	0.00	0.57	-	-	0.22
Sex	3	276.20	0.53	0.43	-	-	0.20
[…]							
Null	2	293.57	17.90	0.00	-	-	-
Shelter change							
Distance water + **Humidity** + Sex + Time btw obs	6	284.68	0.00	0.25	0.16	0.34	-
Distance water + Humidity + Sex + Slope	6	286.14	1.46	0.12	0.15	0.31	-
Distance water + Humidity + Time btw obs	5	286.22	1.54	0.12	0.13	0.32	-
Distance water + Humidity + Sex	5	286.35	1.67	0.11	0.13	0.32	-
Distance water + Humidity + Slope	5	286.91	2.23	0.08	0.13	0.30	-
Distance water + Sex + Time btw obs	5	287.95	3.27	0.05	0.12	0.30	-
Distance water + Humidity	4	288.19	3.51	0.04	0.10	0.31	-
Distance water + Time btw obs + Slope	5	288.30	3.62	0.04	0.12	0.28	-
Distance water + Time btw obs	4	288.46	3.78	0.04	0.10	0.29	-
[…]							
Null	2	297.84	13.16	0.00	0.00	0.24	-

Table C.2 - List of best weighted least square regressions 4<ΔAICc relating spatial ecology metrics of the Asian common toad in Toamasina (Madagascar). Number of parameters estimated in the model (K), AICc score of the model, AICc difference with the best model (ΔAICc), relative weight of the model with respect to the candidate set of models (wAICc), variation explained by the models adjusted for the number of parameters (R2Adj). Only models with 4<ΔAIC and null models are shown. In the best model, significant variables are highlighted in bold.

Response **Model formula**	**K**	**AICc**	**ΔAICc**	**wAICc**	**Adj R^2**
Path straightness					
Null	2	370.03	0	1.00	0
Net displacement					
Tracking time + Temperature	4	338.82	0	0.26	0.06
Temperature	3	338.97	0.15	0.24	0.04
Tracking time	3	338.98	0.16	0.24	0.04
Sex + Rain	4	340.04	1.22	0.14	0.05
Null	2	340.16	1.35	0.13	0.00
Total displacement					
Tracking time	3	337.12	0	0.98	0.11
Null	2	345.18	8.05	0.02	0

Table C.3 - List of weighted least square regressions relating the probability of habitat selection of the Asian common toad in Toamasina (Madagascar). Number of parameters estimated in the model (K), AIC score of the model, AIC difference with the best model (ΔAIC), relative weight of the model with respect to the candidate set of models (*w*AIC), variation explained by the models adjusted for the number of parameters (R^2Adj). Only models with 4<ΔAIC and null models are shown. In the best model, significant variables are highlighted in bold.

Response **Model formula**	**K**	**AIC**	**ΔAIC**	**wAIC**	**Adj R^2**
Habitat characteristics					
Distance cell^2	3	522.24	0.00	1.00	0.62
[…]					
Null	1	1280.97	758.72	0.00	0.00
Habitat dissimilarity					
Distance cell^2 + ΔBarren	4	521.19	0.00	0.63	0.62
Distance cell^2	3	522.24	1.05	0.37	0.62
[…]					
Null	1	1280.97	759.77	0.00	0.00

Appendix C.1

Supervised classification of Sentinel-2 Copernicus satellite imagery

The Sentinel-2 data captured the study area on 07/11/2017 and for the classification all the spectral bands at 10 m spatial resolution (Blue, Green, Red, Near-infrared bands) were considered. The classification considered four land cover classes (forest, shrubland, grassland, and barren land) and a set of 40 training sites.

Validation was performed though a confusion matrix that related reference data (forest, n= 28; shrubland, n= 43; grassland, n= 42; barren, n= 47; water, n= 38) to map data to estimate overall, producers' and users' accuracy. The overall accuracy statistics showed that the map is highly accurate (0.94). The users' accuracy of all land cover classes was in the range 0.90 – 1.0, indicating the high reliability of the map (classes present in the map are actually on the ground). Producers' accuracy values were in the range 0.84 – 1.0, demonstrating the good quality of the map and a slight tendency of the classification to underestimate the areas covered with grass (0.88) and scrub (0.84).

Table C.4. Confusion matrix for all land cover classes

Map/Reference	water	barren	grass	scrub	forest	Producer's accuracy
water	38	0	0	0	0	1.000
barren	0	47	1	0	0	1.000
grass	0	0	37	4	0	0.881
scrub	0	0	4	36	0	0.837
forest	0	0	0	3	28	1.000
User's accuracy	1.000	0.979	0.902	0.900	0.903	

Appendix D

Chapter 6

Article 5

Toad invasion of Malagasy forests triggers severe mortality of a predatory snake

Fig. D.1 - Observations made in Analabe forest: a) snake carcass (arrow) with a dead adult toad (white circle) found less than 1 m apart; b, c) two snakes found dead in the study area without any visible external injuries; d) snake found dead with a subadult Asian common toad in its stomach (e); f) decomposing snake with a subadult toad in its mouth; g) adult Asian common toad visibly wounded in the head, likely the result of a snake predation attempt.

Table D.1 - List of *M. colubrinus* carcasses observed in Analabe forest between December 2018 and May 2019. TL, total length; SVL, Snout-Vent Length.

Date	Location	Observation
06/12/2018*	Analabe, outside study area	ca. 80 cm TL snake with no apparent external injuries with an adult toad approximately 70 cm from its body (Fig. D1a)
23/12/2018*	Analabe, within study area	ca. 50 cm TL snake carcass in advanced state of decomposition
16/01/2019	Analabe, within study area	ca. 60 cm TL snake carcass with dead subadult toad beside its head (Fig. 6.1a)
16/01/2019	Analabe, within study area	ca. 60 cm TL snake found dead with no apparent external injuries (Fig. D1b)
18/01/2019	Analabe, within study area	ca. 60 cm TL snake found dead in a prone position with no apparent external injuries (Fig. D1c)
21/01/2019	Analabe, within study area	ca. 60 cm TL snake found dead with no apparent external injuries
29/01/2019	Analabe, within study area	ca. 80 cm TL snake found dead with no apparent external injuries
14/02/2019	Analabe, within study area	ca. 60 cm TL snake carcass with a subadult toad (ca. 40 mm SVL) inside its stomach (Fig. D1d-e)
15/05/2019	Analabe, within study area	ca. 70 cm TL snake found dead with a subadult toad (ca. 40 mm SVL) in the mouth (Fig. D1f)

Appendix E

Chapter 7

Article 6

Using public surveys to rapidly profile biological invasions in hard-to-monitor areas

Appendix E.1

Questionnaire used to interview local inhabitants in Toamasina province (Madagascar), based on the questionnaire used by (Mohanty and Measey 2019)

1. Have you sighted this animal (photograph of the Asian common toad) in this particular village?
(Ianao ve efa nahita an'io biby io?)

2. Which animal is this (photograph of the Asian common toad)? [verification to be done based on local name and morphology]
(Iza amin'ireto biby ireto ny io biby io?)

If identified correctly,
Structured
3. When did you first see the invasive toad in this village?
(Ovina no nahitanao azy voalohany?)

4. Do you kill the invasive toad for any reason?
(Mamono radaka boka ve ianao?)

Semi-structured
5. Do you incur any benefit due to the invasive toad's presence in this village? If yes, please explain the benefit.
(Nahatsapa vokatsoa avy amin'ny radaka boka ve ianao?)

6. Do you incur any loss due to the invasive toad's presence in this village? If yes, please explain the loss.
(Nahatsapa vokadratsy avy amin'ny radaka boka ve ianao?)

Table E.1 - List of localities assessed in the analysis of the spatiotemporal dynamics of the Asian common toad invasion of Madagascar.

Date	Locality	Latitude	Longitude	Source
07/05/2014	Ambodisaina 1	-18.13924	49.36588	Moore et al., 2015
07/05/2014	Ambodisaina 2	-18.13639	49.3642	Moore et al., 2015
08/05/2014	Farafaty 1	-18.13583	49.36238	Moore et al., 2015
08/05/2014	Farafaty 2	-18.13654	49.3598	Moore et al., 2015
09/05/2014	Antsarimasina	-18.20013	49.33935	Moore et al., 2015
09/05/2014	Tetezantona	-18.15331	49.35891	Moore et al., 2015
12/05/2014	Ambodisaina 3	-18.1397	49.37049	Moore et al., 2015
12/05/2014	Barikadimy	-18.13575	49.37627	Moore et al., 2015
12/05/2014	Mangarano 11/47	-18.13394	49.38105	Moore et al., 2015
14/05/2014	Mahasoa 1	-18.22894	49.32541	Moore et al., 2015
14/05/2014	Mahasoa 2	-18.22573	49.33135	Moore et al., 2015
14/05/2014	Mahasoa 3	-18.22988	49.33034	Moore et al., 2015
15/05/2014	Antanimberika	-18.20995	49.31498	Moore et al., 2015
15/05/2014	Sahavakaka	-18.20348	49.32453	Moore et al., 2015
19/05/2014	Verrerie	-18.16331	49.39371	Moore et al., 2015
20/05/2014	Mangarivotra Nord	-18.14989	49.39322	Moore et al., 2015
20/05/2014	Mangarivotra Sud	-18.1514	49.39167	Moore et al., 2015
21/05/2014	Mangarano CEG	-18.13951	49.38381	Moore et al., 2015
23/05/2014	Tanandava Ambodinonoka	-18.17166	49.32378	Moore et al., 2015
01/07/2014	Ambodibonara 1	-18.1398	49.34828	Moore et al., 2015
07/07/2014	Tsarasaotra	-18.20681	49.31201	Moore et al., 2015
08/07/2014	Ampasimagneva 1	-18.22694	49.34194	Moore et al., 2015
08/07/2014	Ampasimagneva 2	-18.22693	49.35758	Moore et al., 2015
08/07/2014	Ampasimagneva 3	-18.22745	49.36204	Moore et al., 2015
09/07/2014	Antsagabato	-18.24088	49.32694	Moore et al., 2015
09/07/2014	Atserangapetsa	-18.24359	49.33743	Moore et al., 2015
09/07/2014	Mahatsara Fonkontany 1	-18.24733	49.33882	Moore et al., 2015
09/07/2014	Mahatsara Pont Machine	-18.23456	49.32411	Moore et al., 2015
11/07/2014	Antanambao	-18.23921	49.31663	Moore et al., 2015
11/07/2014	Masiakamboay	-18.2422	49.32	Moore et al., 2015
08/10/2014	Ambodizarina 1	-18.14052	49.31113	Moore et al., 2015
08/10/2014	Ambodizarina 2	-18.14044	49.31727	Moore et al., 2015
09/10/2014	Ambalarondra 1	-18.1496	49.34491	Moore et al., 2015
09/10/2014	Ambalarondra 2	-18.1535	49.34232	Moore et al., 2015
13/10/2014	Port fluvial	-18.16578	49.40114	Moore et al., 2015
14/10/2014	Ambony Lavoi 1	-18.26364	49.34023	Moore et al., 2015
14/10/2014	Ambony Lavoi 2	-18.25271	49.33653	Moore et al., 2015
14/10/2014	Canal de Pangalane	-18.18816	49.37544	Moore et al., 2015
14/10/2014	EPP Mahatsara	-18.25386	49.33136	Moore et al., 2015
14/10/2014	Mahatsara Fonkontany 2	-18.24627	49.33915	Moore et al., 2015
14/10/2014	PontAmbatovy	-18.2055	49.3699	Moore et al., 2015
14/10/2014	Unknown3	-18.13043	49.37845	Moore et al., 2015
15/10/2014	Ambatavia	-18.19786	49.29832	Moore et al., 2015
15/10/2014	Vohitaiza	-18.26231	49.32789	Moore et al., 2015
18/10/2014	Ambokarivo	-18.28533	49.32468	Moore et al., 2015

03/11/2014	Morafeno 1	-18.14626	49.30676	Moore et al., 2015
03/11/2014	Morafeno 2	-18.15126	49.30281	Moore et al., 2015
15/11/2014	Antsapanana Colas	-18.211	49.31252	Moore et al., 2015
01/12/2016	Ivoloina	-18.0673	49.37822	Goodman et al., 2017
01/12/2016	Mpangalane	-18.10742	49.39812	Goodman et al., 2017
01/12/2016	Barikadimy South	-18.14385	49.37114	Goodman et al., 2017
01/12/2016	Tsararivotra	-18.1627	49.36166	Goodman et al., 2017
01/12/2016	Antanandava	-18.17324	49.35484	Goodman et al., 2017
01/12/2016	Antsarakofafa	-18.18307	49.36776	Goodman et al., 2017
01/12/2016	NearAntavibe	-18.19698	49.36926	Goodman et al., 2017
01/12/2016	Amboakarivo	-18.22581	49.34243	Goodman et al., 2017
02/11/2016	Tranomora	-18.15055	49.37145	Licata et al. 2019
04/11/2016	Vohitrambato (eglise)	-18.1221	49.33485	Licata et al. 2019
07/11/2016	Sahavilarna	-18.13866	49.30616	Licata et al. 2019
17/11/2016	Mangarano	-18.14296	49.37916	Licata et al. 2019
22/11/2016	Barikadimy	-18.12826	49.38067	Licata et al. 2019
25/11/2016	Farafaty	-18.14259	49.35754	Licata et al. 2019
29/11/2016	Antanimarina	-18.12712	49.36129	Licata et al. 2019
30/11/2016	Mahasoa	-18.23097	49.30517	Licata et al. 2019
08/12/2016	Ambodibonara	-18.13691	49.3499	Licata et al. 2019
09/12/2016	Amparihilava	-18.16865	49.36865	Licata et al. 2019
21/12/2016	Hameau 11-B	-18.11206	49.32826	Licata et al. 2019
21/12/2016	Ambodimangabe	-18.06105	49.18599	Licata et al. 2019
22/12/2016	Antanamakoa	-18.08189	49.17393	Licata et al. 2019
22/12/2016	Ambodizarina	-18.13875	49.29425	Licata et al. 2019
22/12/2016	Ambatofotsy	-18.08038	49.17038	Licata et al. 2019
22/12/2016	Ambodilazana	-18.14083	49.31757	Licata et al. 2019
22/12/2016	Ambodisaina	-18.13951	49.32941	Licata et al. 2019
12/01/2017	Fanandrana	-18.25356	49.26787	Licata et al. 2019
12/01/2017	Ambalanaomby	-18.21338	49.28804	Licata et al. 2019
12/01/2017	Vohilava carriere	-18.20841	49.28598	Licata et al. 2019
12/01/2017	Ampangadiampasika	-18.15128	49.16043	Licata et al. 2019
12/01/2017	Antananambo	-18.15482	49.17166	Licata et al. 2019
20/01/2017	Piste Anjinjanaomby	-18.18676	49.29616	Licata et al. 2019
29/01/2017	Analabemazava	-18.05477	49.3196	Licata et al. 2019
01/02/2017	Tapakala	-18.22797	49.36343	Licata et al. 2019
01/02/2017	Amboditandroho	-18.1623	49.20337	Licata et al. 2019
01/02/2017	Andranompanihy Manitra	-18.28532	49.3367	Licata et al. 2019
01/02/2017	Ampasindava	-18.19063	49.18179	Licata et al. 2019
01/02/2017	Vohiteza	-18.26175	49.32847	Licata et al. 2019
01/02/2017	Ambodisiny	-18.15515	49.21234	Licata et al. 2019
01/02/2017	Tapakala	-18.13407	49.21483	Licata et al. 2019
01/02/2017	Sahafosa	-18.17349	49.17239	Licata et al. 2019
01/02/2017	Vohimarina	-18.306	49.31944	Licata et al. 2019
03/02/2017	Marotanindrazana	-18.30584	49.25178	Licata et al. 2019
15/02/2017	Ankirihiry parcelle 44/50-1	-18.1455	49.39529	Licata et al. 2019
16/02/2017	Pont verrerie	-18.16251	49.39363	Licata et al. 2019

16/02/2017	Morarano parcelle 21/62-63	-18.15623	49.39114	Licata et al. 2019
16/02/2017	Morarano parcelle 21/61	-18.15558	49.39036	Licata et al. 2019
16/02/2017	Pontambalakisoa	-18.15575	49.38993	Licata et al. 2019
16/02/2017	Morarano parcelle 21/43	-18.15298	49.39144	Licata et al. 2019
16/02/2017	Pont Ambolomadinika	-18.15085	49.39286	Licata et al. 2019
17/02/2017	Depot parcelle 23/44	-18.17445	49.39528	Licata et al. 2019
17/02/2017	Tanamborizano parcelle 22/22-23	-18.16488	49.39598	Licata et al. 2019
17/02/2017	Tanamborizano parcelle 22/31	-18.16521	49.39559	Licata et al. 2019
17/02/2017	Tanamborizano Canal	-18.16679	49.39446	Licata et al. 2019
17/02/2017	Tanamborizano parcelle 22/32	-18.16662	49.39437	Licata et al. 2019
15/06/2017	Vohidrotra 1	-18.06078	49.40644	Licata et al. 2019
15/06/2017	Vohidrotra 2	-18.06175	49.39456	Licata et al. 2019
30/09/2017	Secrimad	-18.12619	49.36214	Licata et al. 2019
09/11/2018	Ampiranambo	-18.304771	49.257794	Randriamoria et al., 2019
12/11/2018	Ambavarano	-18.314118	49.314341	Randriamoria et al., 2019
13/11/2018	Andovilalana	-18.314583	49.31332	Randriamoria et al., 2019
13/11/2018	Ampasindava	-18.321154	49.296778	Randriamoria et al., 2019
01/04/2020	Analalava	-17.698263	49.472285	This study

Table E.2 - List of weighted linear regressions relating the year of establishment of the invasive toad with the selected independent variables. The independent variables selected are: distance from the introduction point ("Distance"), distance from the highway ("Road"), direction from the introduction point ("Direction") and community type ("Community").

Model	**d.f.**	**AICc**	**ΔAICc**	**AICc weight**
Distance*	3	125.9	0.0	0.99
Road + Direction	4	182.3	56.4	< 0.0001
Road	3	190.3	64.4	< 0.0001
Direction + Community	4	196.8	70.9	< 0.0001
Direction	3	197.3	71.4	< 0.0001
Null	2	198.3	72.3	< 0.0001

* Best model

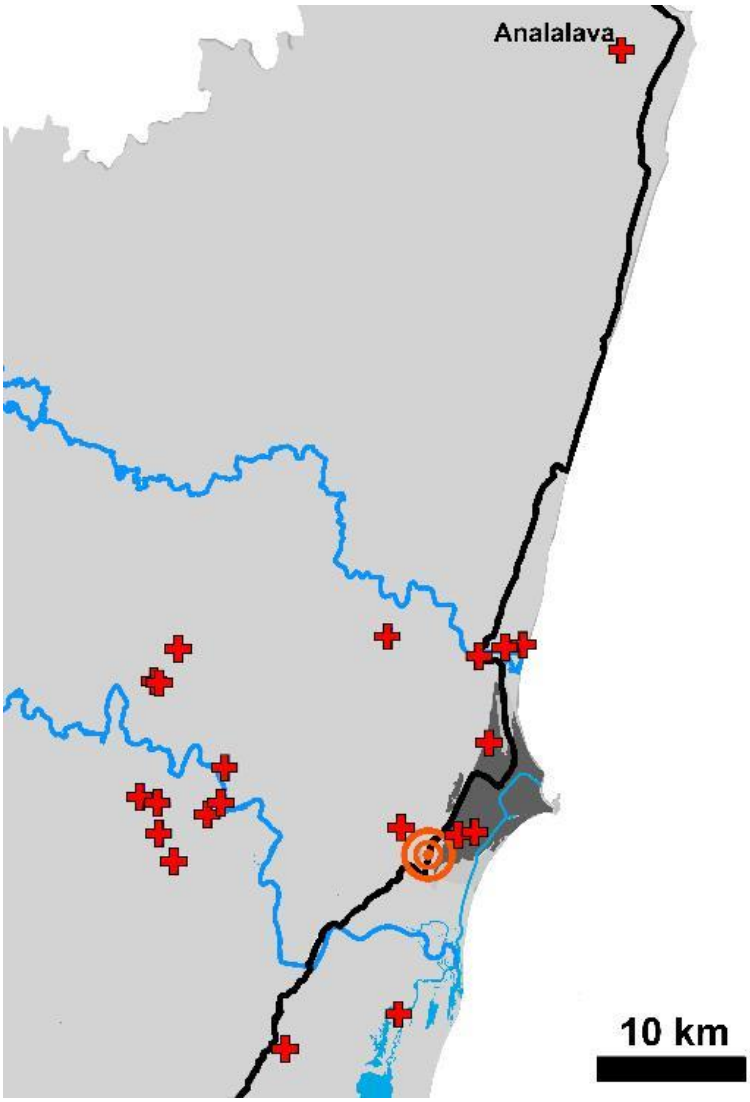

Fig. E.1 - Outlier localities identified using deviation from model expectations. Localities where the establishment of the species likely did not follow the normal leading-edge dispersal but could be attributed either to long-distance or human-assisted dispersal.

Table E.3 -The list and coordinates of the localities for which the observed time of establishment was below the threshold value is also provided.

Locality	Predicted time of arrival (± SE)	Latitude	Longitude	Observed time of arrival	Distance from introduction point	Difference predicted - observed	Reference
Ambokarivo	2016.9 ± 0.1	-18.285	49.325	2014.8	10606.5	2.1	Moore et al., 2015
Ivoloina	2018.7 ± 0.2	-18.067	49.378	2016.9	14202.4	1.8	Goodman et al., 2016
Analalava	2039.6 ± 1.7	-17.698	49.472	2020.7	56239.7	18.9	This study
Ambodisiny	2018.8 ± 0.2	-18.155	49.212	2017.1	14369.5	1.7	Licata et al., 2019
Tapakala	2019.1 ± 0.2	-18.134	49.215	2017.1	14943.2	2.0	Licata et al., 2019
Amboditandroho	2019.2 ± 0.2	-18.162	49.203	2017.1	15096.7	2.1	Licata et al., 2019
Analabemazava	2019.3 ± 0.3	-18.055	49.320	2017.1	15286.2	2.2	Licata et al., 2019
Vohidrotra2	2019.3 ± 0.3	-18.062	49.395	2017.5	15324.4	1.8	Licata et al., 2019
Vohidrotra1	2019.6 ± 0.3	-18.061	49.406	2017.5	15915.0	2.1	Licata et al., 2019
Marotanindrazana	2019.6 ± 0.3	-18.306	49.252	2017.1	15940.1	2.5	Licata et al., 2019
Ampasindava	2020.1 ± 0.3	-18.191	49.182	2017.1	17039.4	3.0	Licata et al., 2019
Sahafosa	2020.7 ± 0.3	-18.173	49.172	2017.1	18139.2	3.6	Licata et al., 2019
Antananambo	2020.9 ± 0.4	-18.155	49.172	2017.0	18552.0	3.8	Licata et al., 2019
Ampangadiampasika	2021.5 ± 0.4	-18.151	49.160	2017.0	19797.2	4.5	Licata et al., 2019
Antanamakoa	2022.4 ± 0.5	-18.082	49.174	2017.0	21578.5	5.4	Licata et al., 2019
Ambodimangabe	2022.6 ± 0.5	-18.061	49.186	2017.0	21971.1	5.6	Licata et al., 2019
Ambatofotsy	2022.6 ± 0.5	-18.080	49.170	2017.0	21982.9	5.6	Licata et al., 2019
Amparihilava	2013 ± 0.3	-18.176	49.364	2009.5	2758.0	3.5	This study
Maison_rouge-Ambodinonoka_I	2013 ± 0.3	-18.171	49.327	2010.0	2747.0	3.0	This study
Tsarahonenana	2013.6 ± 0.2	-18.174	49.374	2012.0	3822.0	1.6	This study
Ambalamanasy_C1	2016.1 ± 0.1	-18.120	49.384	2014.5	8960.0	1.6	This study

Table E.4 - List of models predicting the occurrence of the Asian common toad at 79 localities in Toamasina province. The table reports the list of models ranked by AIC, with estimates of occupancy(ψ), true positive detection probability (*p11*), and false positive detection probability (*p10*) along with 95% confidence intervals.

Model	**AIC**	**ΔAIC**	**AIC weight**	**Occupancy (ψ)**	**True-positive (*p11*)**	**False-positive (*p10*)**
ψ(Distance + Direction), p(.), p10(.), b(.)	964.2	0.0	0.6	site-specific	0.93 (0.91–0.94)	0.01 (0.004–0.03)
ψ(Distance + Road), p(.), p10(.), b(.)	965.8	1.7	0.3	site-specific	0.93 (0.91–0.94)	0.01 (0.004–0.03)
ψ(Distance), p(.), p10(.), b(.)	966.7	2.6	0.2	site-specific	0.93 (0.91–0.94)	0.01 (0.004–0.03)
ψ(Community), p(.), p10(.), b(.)	1000.2	36.0	0.0	site-specific	0.93 (0.91–0.94)	0.01 (0.004–0.03)
ψ(Direction), p(.), p10(.), b(.)	1010.8	46.6	0.0	site-specific	0.93 (0.91–0.94)	0.01 (0.004–0.03)
ψ(.), p(.), p10(.), b(.)	1015.6	51.4	0.0	0.94 (0.77–0.98)	0.93 (0.91–0.94)	0.01 (0.004–0.03)

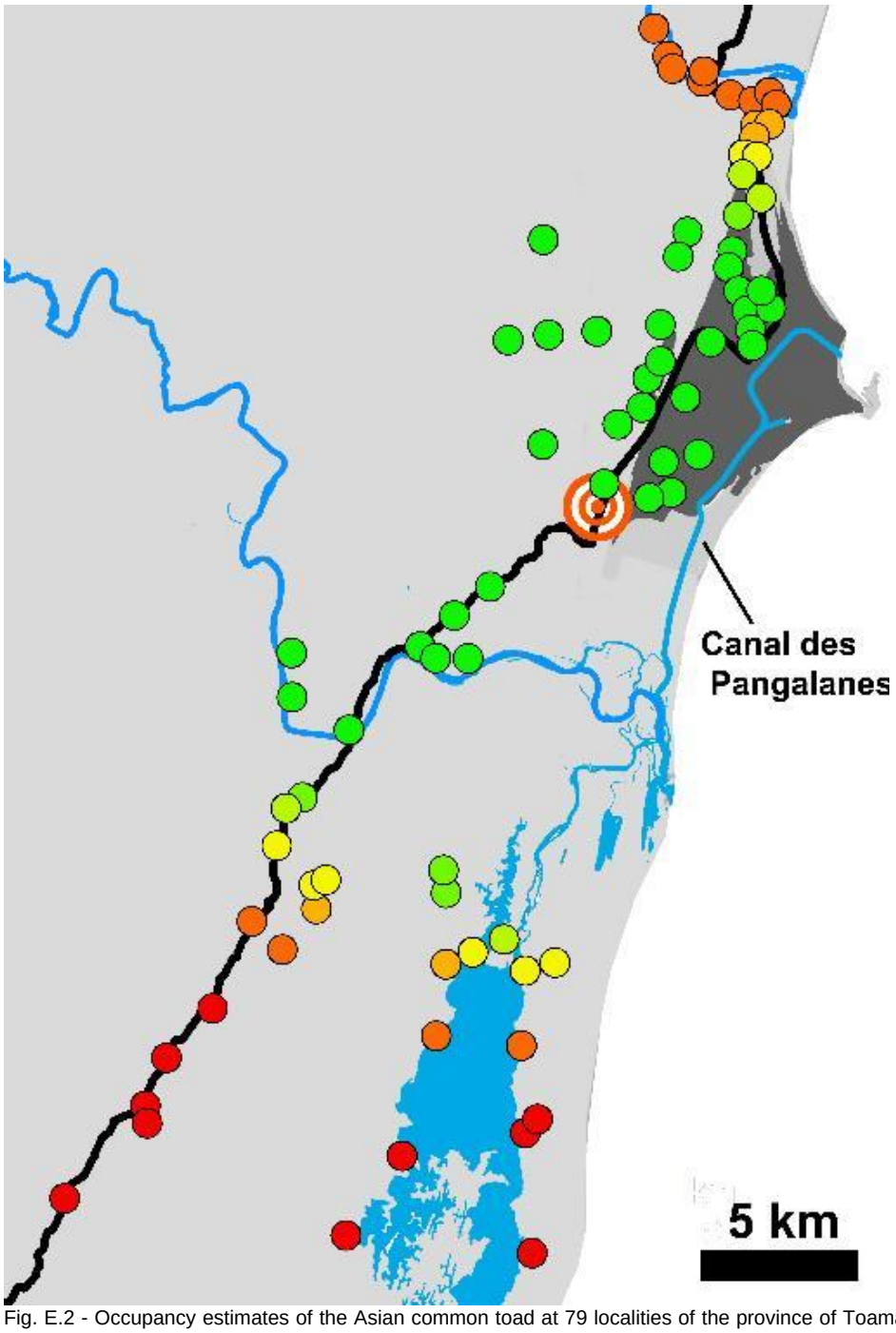

Fig. E.2 - Occupancy estimates of the Asian common toad at 79 localities of the province of Toamasina. Colour gradient (red to green) indicates increasing occupancy rates (0–1), the black line represents the road Route Nationale 2. The best model predicts decreasing occupancy moving away from the introduction point (orange bull's eye), with comparatively higher occupancy estimates in the southern front of the invasion, over the same distance.

Table E.5 - List of binomial GLMMs relating the drivers of public perception and attitude towards the Asian common toad. The independent variables listed are: community type ("Community"), distance from the introduction point ("Distance"), "Gender" and "Age" of the respondent.

Response category	Independent variables	d.f.	AIC	ΔAIC	AIC weight
Negative					
	Distance + Community	4	740.11	0	0.994
	Distance	3	750.41	10.29	0.006
	Community	3	758.89	18.77	< 0.0001
	Null	2	763.83	23.72	< 0.0001
Kill					
	Gender + Distance	4	808.16	0	0.688
	Gender	3	810.08	1.92	0.266
	Distance	3	814.2	6.03	0.031
	Null	2	815.93	7.75	0.014
Nuisance					
	Distance + Community	4	515.03	0	0.78
	Distance	3	517.63	2.59	0.21
	Community	3	525.69	10.66	<0.01
	Null	2	526.26	11.22	<0.01
Health threat					
	Gender	3	605.93	0	0.25
	Distance + Gender	4	605.93	0.005	0.25
	Distance	3	605.99	0.06	0.24
	Null	2	606.08	0.92	0.23
Ecosystem threat					
	Community + Gender	4	274.92	0	0.68
	Community	3	277.65	2.73	0.17
	Gender	3	278.31	3.39	0.12
	Null	2	281.97	7.05	0.02
Economic threat					
	Gender + Age	4	452.96	0	0.42
	Gender	3	454	1.03	0.25
	Age	3	454.24	1.27	0.22
	Null	2	455.89	2.93	0.09

Table E.6 - Best binomial GLMMs relating the drivers of public perception and attitude towards the Asian common toad. For each response category is shown the significance of variables included in the best AIC model. In bold, the significant variables.

Response category	Variable	β	SE	*Z*	*p*-value
Negative					
	Community (Urban)	-1.468	0.411	-3.568	**< 0.001**
	Distance	-0.951	0.207	-4.59	**< 0.001**
Kill					
	Gender (Man)	0.515	0.181	2.843	**0.004**
	Distance	0.23	0.114	2.018	**0.043**
Nuisance					
	Community (Urban)	-1.451	0.656	-2.211	**0.027**
	Distance	-0.989	0.272	-3.633	**< 0.001**
Health threat					
	Gender (Man)	-0.329	0.225	-1.46	0.144
Ecosystem threat					
	Community (Urban)	-1.536	0.627	-2.452	**0.014**
	Gender (Man)	0.792	0.371	2.134	**0.032**
Economic threat					
	Gender (Man)	0.478	0.264	1.806	0.07
	Age	0.232	0.134	1.724	0.084

Appendix E.2

Potential factors involved in the limited dispersal rate of Asian common toad

Asian common toads are smaller and have significantly shorter hind limbs than cane toads, which may result in a comparatively diminished locomotor performance that can partially explain the observed difference in invasion speed (Enriquez-Urzelai et al. 2015; Cayuela et al. 2020). The limited invasion speed could be also due to ontogenetic differences in spread rates, where juveniles are possibly more dispersive than adults (e.g., Cayuela et al. 2019). Morphological analyses suggest that this process might take place in Madagascar (Licata et al. 2021), and is also supported by several anecdotal reports of juvenile toads outnumbering adults in recently invaded localities (AC, FL; pers. observ.). Other limiting factors include sex-biased dispersal (e.g.,(Lampert et al. 2003), which could lead to reduced densities at the invasion front affecting the mate-finding mechanism and triggering an Allee effect (Taylor and Hastings 2005), and periodic or stochastic environmental fluctuations, which may affect population growth rates and dispersal (Neubert et al. 2000). Lastly, the relatively slow rate of spread may be due to limited intrinsic dispersal capacity of the species (Hui and Richardson 2017), whose spatial ecology still needs to be thoroughly investigated.

Appendix E.3

Potential determinant factors for the occurrence of the Asian common toad

The occupancy rates of the Asian common toad are higher in the southern portion of the invasive range (Fig. E.1). This may be due to the presence of the Canal des Pangalanes, a network of man-made, lentic waterways which could have facilitated the invasion by providing suitable breeding habitat to the species (Moore et al. 2015; Licata et al. 2019), or might have provided the opportunity for human-mediated accidental dispersal (at least one locality along its shores is a candidate for invasion *via* jump dispersal; Fig. E.2). Either way, this waterway system represents an important potential dispersal corridor which may seriously jeopardize future management efforts, as it is used for the transport of goods and extends over 600 km along the east coast of Madagascar.

Identifying the categories of goods responsible for the accidental introduction of toads along the Canal des Pangalanes (and elsewhere) may represent a first step to address the problem. Some progresses have been made in this direction of introducing biosecurity measures in Madagascar the (VII IUCN World Conservation Congress 2020), and our study reiterates the urgent need to put such measures in place to avert worst invasive scenarios as already suggested by other authors (Reardon et al. 2018; Randriamoria 2019; Licata et al. 2020),

Appendix E.4

Perceived effects of toads likely resulting from shared narrative about toad's toxicity

The potential toxic effect of toads on human health is among the public's most recurring worries regarding these animals (Table 7.1; Ríos-Orjuela et al. 2020). However, poisoning effects only occur if toads are ingested or if the skin-secreted toxins are absorbed through mucous membranes (Souvannasing et al. 2007; Trainor 2009). This perceived threat may constitute a potential stressor for local communities, as people could avoid areas where toads are abundant, feel uncomfortable at their home, or be deterred from using water sources.

Toad poisoning effects were also reported in pigs (Table 7.1), which, apart from anecdotal reports (e.g.,Lever 2001; Begg et al. 2003), have never been observed feeding on toads, although they are likely sensitive to toad toxins (Shine et al. 2016). Toad-induced decline of snakes can have major cascading effects on ecosystems, including the facilitation of rodent populations (Hill 2008). Therefore, the reported increase of rodents after toad arrival (Table 7.1) can represent a true impact which is worth quantifying.

Impact on domestic apiaries

The Asian common toad prey upon hymenopterans (Döring et al. 2017), and, in Madagascar, private apiaries are often made of makeshift materials, easily accessible by toads, which also have good climbing capacities (Norval et al. 2009). We observed empty apiaries putatively depredated by toads, and directly observed toads gathering around private apiaries to prey on bees at different localities. These observations are substantiated by the loss of commercial apiaries of a local technical support agency in horticulture (i.e., Centre technique horticole de Tamatave, CTHT) (M. Jahiel, priv. comm.).

Appendix E.5

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Appendix F

Thesis chapters published as articles

Biol Invasions
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ORIGINAL PAPER

Abundance, distribution and spread of the invasive Asian toad *Duttaphrynus melanostictus* in eastern Madagascar

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Abstract The Asian toad, *Duttaphrynus melanostictus*, was accidentally introduced to Toamasina (eastern Madagascar) around 2010, and since then has spread at a substantial rate across a larger area. This study documents the expansion of the invasive range of this species, calculates the invasion spread rate, and provides estimates of toad abundance and habitat preferences. Updates of the distribution range revealed a fivefold increase of the invaded area over 3 years, and a doubling of the rate of spread, showing a shift of the invasion towards the North-West, most probably because of the absence of ecological barriers. We used N-mixture models to estimate toad abundance on the basis of repeated count data of six areas in Toamasina and its surrounding countryside. Toad distribution shows heterogeneous density across the distribution range, with an average abundance of 184 toads ha^{-1} (95% CI 132–263). The toad's abundance was highest in sites with the presence of organic waste, and was negatively related to the density of road networks in the proximity of study sites. The rapid expansion of the Asian toad in the Toamasina region suggests that this toad is an increasing threat for Madagascar. We propose immediate management

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Chapter 21
The Asian Toad (*Duttaphrynus melanostictus*) in Madagascar: A Report of an Ongoing Invasion

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21.1 Introduction

The need to identify biodiversity hotspots emerged as a direct consequence of increasing negative anthropogenic effects on ecosystems, driving scientists and experts to pinpoint priorities for conservation actions (Mittermeier et al. 1998; Myers et al. 2000). The 36 world regions currently identified as biodiversity hotspots represent just 2.4% of Earth's land surface although they support more than half of the world's endemic plant species and nearly 43% of all endemic bird, mammal, reptile and amphibian species (Conservation International 2019). Madagascar was classified as one of the richest and most threatened biodiversity hotspots worldwide, with only about 10% of its ecosystems persisting in a pristine or semi-pristine state (Myers et al. 2000; Goodman and Benstead 2005). Madagascar's uniqueness is characterized not only by the total number of species but also by the higher level of phylogenetic distinctiveness of its endemic families and genera (Ganzhorn et al. 2014). For example, among the extant vertebrates, the majority of the lineages succeeded in colonizing Madagascar through sea dispersal, during the Cretaceous – Cenozoic times, when ocean currents were particularly favourable for dispersion

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SHORT COMMUNICATION

Does spatial sorting occur in the invasive Asian toad in Madagascar? Insights into the invasion unveiled by morphological analyses

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Abstract

Dispersal-relevant traits may be subjected to spatial sorting in rapidly expanding populations, driving the evolution of dispersive phenotypes. This process has been largely documented in invasive species, and ascertaining its presence can have important implications for their management. The Asian common toad was accidentally introduced to Madagascar around 2010 and is a highly problematic invasive species. Exploring the morphology of this species offers the twofold opportunity to test the hypothesis of early onset of spatial sorting and to gather information on the dynamics of this unstudied biological invasion. For these purposes, we analyze morphological data of adult toads collected across the invasive range in Madagascar, and we test the effect of the distance from the introduction point on the relative size of head, radioulna and tibiofibula, and on body size and body condition of toads, respectively. We did not detect evidence of spatial sorting in the morphological traits analyzed. Instead, morphological variation was largely dependent on sex and body size of individuals. Sexual dimorphism was limited in relation to other populations in the native range, and body size did not vary across the invasive gradient, which could indicate that both adults and juveniles are currently contributing to the dispersal in this invasive species. We provide important baseline data for the long-term assessment of morphological variation in the invasive population, and we advocate for further investigations of spatial sorting in life history and behavioral traits.

KEYWORDS
dispersal-relevant traits, *Duttaphrynus melanostictus*, invasion dynamics, spatial sorting, Toamasina

Riassunto

Nelle popolazioni in rapida espansione i tratti rilevanti per la dispersione possono essere soggetti a spatial sorting, portando all'evoluzione di fenotipi dispersivi. Questo

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ORIGINAL PAPER

Toad invasion of Malagasy forests triggers severe mortality of a predatory snake

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Abstract The Asian common toad *Duttaphrynus melanostictus* has been introduced to the eastern province of Toamasina in Madagascar, where it is feared to be having devastating effects on native communities by poisoning frog-eating predators. So far it is unclear whether the toad can invade forest habitats, and empirical evidence of its impact on native predators is lacking. We used radio tracking to investigate the spatial behaviour of adult toads in a small parcel of lowland humid forest and we quantify the disruptive effects of toad poisoning on a native frog-eating snake, the Malagasy cat-eyed snake (*Madagascarophis colubrinus*). We used *N*-mixture models to estimate the population size of cat-eyed snakes, and used the mortality events recorded in the same area to obtain an estimate of monthly mortality rate of snakes due to toad poisoning. Our results point to a drastic mortality rate of snakes that, if constant through the year, could halve the predator population, with the potential risk of local extirpation. We expect cat-eyed snake populations across rural and suburban areas of Toamasina to be severely affected by the toad invasion, and suggest that future research should

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