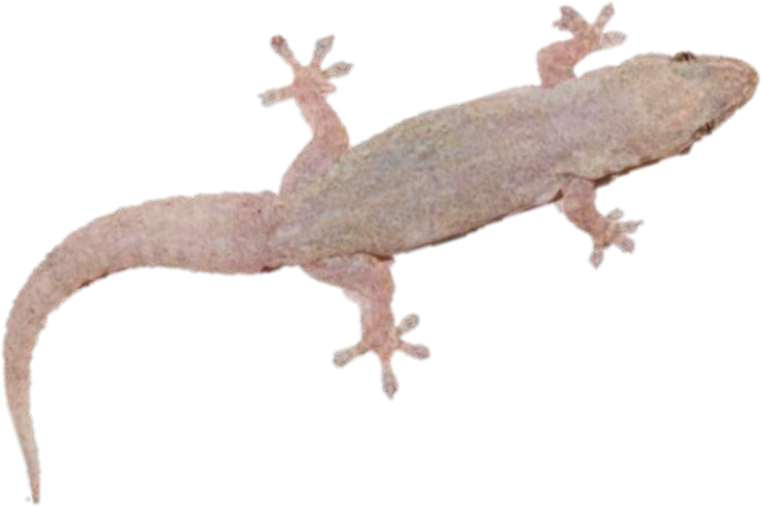
Barnett, Louise Katherine (2017) ***Understanding range expansion of Asian house geckos (Hemidactylus frenatus) in natural environments.*** **PhD thesis, James Cook University.**

Understanding range expansion of Asian house geckos (*Hemidactylus frenatus*) in natural environments



Thesis submitted by
Louise Katherine Barnett
BSc (Hons)
October 2016

Revised thesis submitted March 2017

For the degree of Doctor of Philosophy
College of Science and Engineering
James Cook University
Townsville, Queensland 4811
Australia

Front cover: Asian house gecko (*Hemidactylus frenatus*). Photo by Stephen Zozaya.

Acknowledgements

This project has been a huge challenge for me, but it has also been an awesome adventure, largely due to the wonderful people I met and worked with along the way.

Firstly, an enormous thank you to my advisors Conrad Hoskin and Ben Phillips for your guidance, encouragement, support and feedback throughout the entire project. I couldn’t have done it without you. Conrad, thank you for taking me under your wing and giving me a glimpse of how an expert herpetologist works. Without your training there may not have been any geckos featured in this thesis! Ben, thank you so much for introducing me to the wonderful world of R, and for imparting some of your stats-wizardry. I have a long way to go, but I am definitely hooked.

This project involved a lot of fieldwork and would not have been possible without all the volunteers and friends who braved mosquitoes, stray dogs and brown snakes to search for geckos with me. A massive thank you to: James Murray, Maddie Wuth, Margaret Murray, Michael Lee, Stewart Macdonald, Sheryn Brodie, Kelsey Hosking, Stephen Zozaya, Roderick Budd, Kirilly O’Reilly, Dylan Tegtmeier, Kyana Pike, Nantida Sutummawong, Theresa Rüger, Nadiah Roslan, Matthew McIntosh, Legana Fingerhut, Yan Ow, Tiffany Sih, Steven Johnson, Lauren Heilbronn, Jen Atherton, Hector Barrios, Eric Nordberg, Mat Vickers, Seun-Jin Hong, Natalie Wildermann, Kelsey Miller, Jenna Wanamaker, Isabel Ender, Gina Barnett, Eric Vanderduys, April Reside, Betsy Roznik, Amy Thompson, Rebecca Exl, Alana Trott, Helios Martinez, Russell Stanford and Abigail Nelson.

Thanks to Andrew Coates for being an excellent ‘parasite buddy’ in the field and lab, and to Megan Higgie and Allen Heath for your expert advice and collaboration on papers. Thanks also to the members of the Adaptation Lab and the Centre for Tropical Biodiversity and Climate Change for your feedback on seminar presentations and moral support.

To my officemates, especially Stewart Macdonald, Stephen Zozaya, Nantida Sutummawong and Nadiah Roslan, thank you for the fun times herping, the coffee breaks, the pancake parties, the chocolate eating and the Miley Cyrus-ing. Also, thanks Stephen for letting me use your photos in this thesis.

To my other wonderful friends/second family in Townsville, thanks for your friendship, laughter and for making it feel like home. To my actual family, especially my mum, dad, and Sam – thank you for your unwavering encouragement, support and belief in me.

Last, but most definitely not least, to James Murray, thank you for being the *best*.

Louise Barnett

October 27 2016

Publications associated with thesis

This thesis has been prepared as a series of manuscripts that have either been published or prepared for publication. Each manuscript has been formatted in the style of the target journal. This thesis also includes an Introduction (Chapter 1) and a Thesis Summary (Chapter 6). The below table provides details of author contributions for each manuscript.

Chapter	Publication	Status	Statement of authorship
2	Barnett, L.K., B.L. Phillips and C.J. Hoskin (2016) Going feral: Time and propagule pressure determine range expansion of Asian house geckos into natural environments. *Austral Ecology*	Published	LKB collected the data. LKB, CJH and BLP conceived the analyses. LKB performed the analyses. LKB wrote the manuscript. CJH and BLP edited the manuscript. All authors contributed to ideas and revisions.
3	Barnett, L.K., M. Higgie, B.L. Phillips and C.J. Hoskin (in prep) Morphological divergence in woodland populations of a human-associated invasive gecko. *Biological Invasions*	In preparation	LKB collected the data. LKB, MH and CJH conceived the analyses. LKB performed the analyses. LKB wrote the manuscript. CJH, BLP and MH edited the manuscript. All authors contributed substantially to ideas and revisions.
4	Barnett, L.K., B.L. Phillips, A. Heath, A. Coates and C.J. Hoskin (in prep) Host specificity and the potential impact of parasites during range expansion of an invasive gecko. *OIKOS*	In preparation	LKB conducted the surveys. LKB, AC and CJH collected specimens, AH identified the specimens. LKB, CJH and BLP conceived the analyses. LKB conducted the analyses. LKB wrote the manuscript. CJH, BLP, AH and AC edited the manuscript.
5	Barnett, L.K., C.J. Hoskin and B.L. Phillips (in prep) Modeling individual-level detection probability of invasive Asian house geckos in natural environments *Biological Invasions*	In preparation	LKB collected the data. BLP conceived the analyses. LKB performed the analyses. LKB wrote the paper. CJH and BLP edited the paper. All authors contributed substantially to ideas and revisions.

Statement of contribution of others

Research funding and stipend

- Australian Postgraduate Award
- Australian Geographic
- James Cook University Centre for Tropical Biodiversity and Climate Change

Supervision

- Dr Conrad Hoskin, Centre for Tropical Biodiversity and Climate Change, JCU
- Dr Ben Phillips, Centre for Tropical Biodiversity and Climate Change, JCU & University of Melbourne

Co-authorship of published manuscripts

Co-authors of published manuscripts within this thesis participated in one or more of the following ways: discussions of concept and study design, advice on methods and editing of manuscripts, collation and/or generation of data.

Every reasonable effort has been made to gain permission and acknowledge the owners of copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged.

Animal ethics statement

The research presented and reported in this thesis was conducted in compliance with the National Health and Medical Research Council (NHMRC) Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 7th Edition, 2004 and the Qld Animal Care and Protection Act, 2001. The proposed research study received animal ethics approval from the JCU Animal Ethics Committee (approval number A1801).

Abstract

Over the past 50 years accidental introductions of invasive species have accelerated due to globalisation and increased transit efficiency. Once established, invasive species may affect biodiversity through predation, competition or hybridisation with native taxa, or through the introduction of novel parasites. The impacts of invasive species are mediated by their distribution within the introduced range, and it is important to understand the processes controlling range expansion of invasive species. Human-associated invasive species are often overlooked as potential threats to native species in natural environments, due to their perceived restriction to anthropogenic habitats. It is, nonetheless, likely that some of these species will eventually spread into natural habitats due to their broad global distributions, propensity to establish large populations in urban areas and the ever-expanding reach of human infrastructure. Human-associated species also present ideal systems to assess the factors driving range expansion across abrupt ecological boundaries because of the stark differences between urban areas and surrounding natural environments, and because urban areas often provide multiple semi-independent invasion fronts to compare within the same geographic region.

This thesis investigates the factors that facilitate range expansion of a human-associated invasive species, the Asian house gecko, *Hemidactylus frenatus,* into natural habitats in the Townsville region of northern Australia. It outlines how demographic factors, environmental factors, morphological divergence and parasitism affect range expansion, touches on the potential impacts of *H. frenatus* in these environments and establishes an effective method for *H. frenatus* detection.

Chapter 2 investigates whether there are established populations of *H. frenatus* in natural environments, and assesses whether environmental or demographic factors affect expansion into natural habitats. This was achieved by surveying ten transects in the Townsville region of north Queensland every month for a year. Each transect was made up of five sites positioned every 500 m from the urban edge up to 2 km into surrounding woodland. This work revealed that the most important factors facilitating expansion into woodland environments are propagule pressure (i.e., relative abundance of *H. frenatus* at the urban edge) and the number of years since *H. frenatus* established in the urban area. Environmental factors, such as coarse habitat structure and size of the urban area did not affect range expansion.

Chapter 3 examines whether morphological divergence occurs during expansion of *H. frenatus* into natural environments across multiple semi-independent invasion fronts. *Hemidactylus frenatus* were collected from paired urban and woodland sites for assessment of body and toe morphology. Results indicate that *H. frenatus* have morphologically diverged in natural environments, but the direction of some trait shifts differed between the three semi-independent invasion fronts studied.

Chapter 4 investigates the potential impact of parasites during range expansion of *H. frenatus* into natural environments, as well as the potential for parasite transmission between *H. frenatus* and co-occurring native geckos. There was no evidence for transmission of native or invasive *Geckobia* mites between *H. frenatus* and native geckos; however, in this system *H. frenatus* can host native Australian pentastomes of the *Waddycephalus* genus. The relatively high density of *H. frenatus* in natural habitats makes parasite spillback of *Waddycephalus* to native host species a concern.

Having demonstrated that *H. frenatus* can establish large populations in natural environments, it is important to understand the most effective technique for early detection of this species. **Chapter 5** uses data collected over multiple site visits to investigate which abiotic factors affect individual-level detection probability of *H. frenatus*. Binomial mixture models are used to assess the optimal conditions for detecting *H. frenatus* in natural environments and to compare the efficiency of listening and visual surveys. I found that multiple site visits are necessary for detection of this species. However, detection probability can be maximised by choosing to survey in appropriate conditions, and through pairing five-minute listening surveys and 15-minute visual searches.

This thesis assesses a number of factors known to affect range expansion of invasive species and investigates their importance in relation to the establishment of Asian house geckos in natural environments. It highlights the complex nature of range expansion across abrupt habitat boundaries and demonstrates that human-associated species can provide ideal systems to investigate range expansion and rapid adaptive responses.

Table of contents

Acknowledgements…………………………………………………………..… iii

Publications associated with thesis……………………………………….. v

Statement of contribution of others…………………………………..… vi

Animal ethics statement ………………………………………………..… vi

Abstract………………………………………………………………...…. vii

Table of contents………………………………………………………….. x

1 – Introduction……………………………………………………….... 1

2 – Going feral: Time and propagule pressure determine range expansion of Asian house geckos into natural environments………………………. 25

3 – Morphological divergence in woodland populations of a human-associated invasive gecko ……………………………………..… 45

4 – Host specificity and the potential impact of parasites during range expansion of an invasive gecko………………………………….. 89

5 – Modeling individual-level detection probability of invasive Asian house geckos in natural environments……………………………. 123

6 – Thesis summary…………………………………………………….. 155

Chapter 1.

Introduction

Range expansion of invasive species

Invasive species cause extensive ecological and economic damage, and pose a significant threat to biodiversity on a global scale (Mack et al. 2000, Russell and Blackburn 2016). Some invasive species are deliberately introduced outside their native range (e.g., biological control animals, pets and agricultural species) and subsequently escape confinement, while others are accidentally introduced through human transport networks (e.g., pest species, such as rats and cockroaches) (Hulme 2009, Consuegra et al. 2011). Once established, invasive species may affect biodiversity through predation, competition or hybridisation with native taxa, or through the introduction of novel parasites and pathogens (Wanless et al., 2007; Andreou et al., 2012; Short and Petren, 2012). These pressures on native species can lead to local extirpations, extinctions, or even ecological collapse (O'Dowd et al. 2003). Accidental introductions of invasive species have accelerated over the last 50 years due to globalisation and increased transit efficiency (Richardson and Ricciardi 2013), and invasive species are currently recognised as one of the greatest threats to global biodiversity (Russell and Blackburn 2016).

Studying biological invasions is undoubtedly critical for conservation; however, introduced organisms also present important systems to assess range expansion (Phillips et al. 2007, White et al. 2012). In the current era of global change, understanding the processes that drive range expansion is more important than ever, and range expansion may allow some species to persist under climate change (Fordham et al. 2013, Reside et al. 2014). Invasive species present ideal systems to assess range expansion, as they are often introduced to areas with novel climates, predators and competitors, and adaptations might be necessary to thrive and disperse in these new habitats (Leger and Rice 2007, Urban et al. 2007). Furthermore, because a number of biological invasions have occurred relatively recently (Hulme 2009), many invasive species are yet to achieve their potential distributions within the introduced range, and so provide good systems to assess which factors affect range expansion as it is happening (Urban et al. 2007).

The rate of range expansion in invasive species is difficult to predict and the potential impact of introduced species is often underestimated due to slow initial spread in the new habitat (Crooks 2005, Boggs et al. 2006, Aikio et al. 2010). Some invasive species may remain restricted to a small area of suitable habitat, or exhibit stalled population growth for many years after introduction (Rilov et al. 2004, Crooks 2005). These periods of relatively slow population growth or spread are known as lags (i.e., lag period, lag phase and lag time). Lags are sometimes followed by a rapid increase in range and/or population size, which can take ecologists and land managers by surprise (Crooks 2005). Expansion of invasive species after lag periods may have devastating consequences for native biota and ecosystems (Rilov et al. 2004, Crooks 2005), and it is important to investigate the invasion potential of introduced species, even those that initially appear benign (e.g., human-associated or range-restricted taxa) (Richardson et al. 2008).

Human-associated species

Species that are closely associated with humans may be particularly likely to experience delayed range expansion. Human-associated animal species are commonly introduced to new areas as passengers in human development (Jeschke and Strayer; 2006; Wilson et al., 2009; Chapple et al., 2012) and are often initially restricted to anthropogenic environments in the introduced range. Familiar examples of human-associated species include house sparrows (*Passer domesticus*), pigeons (*Columba livia*), common mynas (*Acridotheres tristis*), house mice (*Mus musculus*), house geckos (e.g., *Hemidactylus frenatus*), and American cockroaches (*Periplaneta americana*) (Keller 2007, Sætre et al. 2012, Martin et al. 2015). These animals thrive in urban areas and have often been living in association with humans for so long that their natural habitat in their native range is poorly known (Hoskin 2011, Sætre et al. 2012).

Despite close ties to humans, it is likely that some human-associated animal species may eventually spread into natural environments. Some human-associated plant species (ornamental and agricultural plants) have been demonstrated to spread into and thrive in natural areas following a lag time (Simberloff et al. 2010, Mukherjee et al. 2011). These plant species share many attributes with human-associated animal species, including broad global distributions, initial reliance on anthropogenic resources, and the ability to grow unnaturally large populations in anthropogenic environments. Nonetheless, little research has assessed what causes expansion of human-associated animals into natural habitats.

Anthropogenic areas differ from surrounding natural environments in a number of ways that might affect range expansion of human-associated species across the urban–natural interface (Winchell et al., 2016; Fig. 1). For example, in urban areas human-associated species often rely on resources that are either directly provided by humans (e.g., food waste; Randa and Yunger, 2006), or are a bi-product of anthropogenic change (e.g. aggregations of insects around artificial lights; Petren and Case, 1998). Urban areas also have different assemblages of predators and competitors to natural habitats, with less biological diversity, but higher abundances of certain species (Savard et al. 2000, Møller 2008). In anthropogenic habitats impervious surfaces provide thermal buffering to the species that live there, whereas the thermal regimes in surrounding natural environments are likely to be much more variable (Lei and Booth 2014). Furthermore, urban areas differ structurally to surrounding natural habitats, and these structural differences can mediate interactions between invasive and native species (Petren and Case 1998). Overall, stark differences between urban and natural habitats mean that range expansion of human-associated species across the urban–natural interface is unlikely to occur immediately after establishment in the urban area.

Figure 1. An example of an (A) urban and a (B) natural environment in northern Australia

What facilitates expansion of human-associated species after a lag period?

Propagule Pressure

Invasion theory tells us that propagule pressure will be particularly important in the range expansion of human-associated species (Gomulkiewicz et al. 1999, Colautti et al. 2006). Propagule pressure is a composite measure of the number of individuals introduced and the number of introduction events (Lockwood et al. 2005). Increasing propagule pressure may relieve Allee effects and facilitate population growth (Lockwood et al. 2005, Simberloff 2009). Human-associated species often build up large populations in urban areas (Shochat et al. 2010), which may result in a large number of individuals entering natural environments (i.e., high propagule pressure). Furthermore, propagule pressure is often associated with increased genetic diversity in invasive populations (Gaither et al. 2012), which may increase the likelihood of adaptive change (Holt et al. 2004).

Adaptations

Adaptations in physiology, behaviour and morphology are common in invasive species and are often associated with success in novel environments (Lee 2002, Prentis et al. 2008, Suarez and Tsutsui 2008). Adaptations can arise rapidly after establishment in novel environments (Losos et al. 1997). For example, field crickets (*Teleogryllus oceanicus*) that were exposed to a novel acoustically oriented parasitoid fly (*Ormia ochracea*) lost the ability to call after only 20 generations (approximately five years) (Zuk et al. 2006).

For species transitioning abrupt habitat boundaries like the urban–natural interface, morphological adaptations may be particularly likely (Winchell et al. 2016). Morphological traits govern how organisms interact with their environment, affecting susceptibility to predation (Kotler et al. 1988, Vervust et al. 2007), thermal tolerance (Wikelski 2005), foraging efficiency and locomotive performance (Irschick and Garland, 2001; Higham and Russell, 2010), and different environments likely favour different body shapes. A recent study documented morphological shifts in the lizard *Anolis cristatellus* upon the transition from natural to urban environments (Winchell et al. 2016), and it is likely that morphological adaptations are also important during colonisation of natural habitats by human-associated animals.

Parasites

Parasites associated with invasive species may affect range expansion and impact native species in the introduced range (Svenning et al. 2014, Dunn and Hatcher 2015). During range expansion, populations on the invasion front often have a lower prevalence of parasites than longer established populations (Phillips et al. 2010), and this parasite release may facilitate further expansion. Conversely, if invasive species are susceptible to native parasites in the introduced range, these native parasites may constrain range expansion into certain habitats (Case and Taper 2000), although empirical evidence of this in natural systems is lacking.

For human-associated species, release from co-evolved invasive parasites is likely to occur during range expansion into natural environments (Coates et al. 2017). The prevalence of native parasites is, however, likely to be greater in natural areas, because there is generally a higher diversity of native species in these habitats (Grimm et al., 2008; Coates et al., in press). Therefore, when investigating the impact of parasites on range expansion of invasive species, it is important to understand the prevalence of both native and introduced parasites and their respective impacts on the invasive host.

As well as affecting range expansion, parasites of invasive species can impact native species in the invaded range. Upon expansion into natural areas, human-associated invasive species are likely to come into contact with a greater diversity of native species (Colla et al. 2006), and it is vital to understand the impact of associated parasites in these systems. Parasites introduced with the invasive species can ‘spillover’ to naïve native hosts, resulting in decreased body condition, reduced fitness or even extinction of native hosts (Andreou et al. 2012, Lymbery et al. 2014). Furthermore, if the invasive species hosts native parasites, parasite ‘spillback’ can occur, whereby the invasive hosts increase the prevalence of native parasites in the environment, resulting in more native individuals being infected (Kelly et al. 2009). Therefore, by investigating parasite dynamics during biological invasions we can increase our understanding of what drives range expansion, and elucidate the potential impact of parasite transmission between invasive and native species.

Detection of invasive species in natural environments

While lag phases, by definition, should represent actual periods of stalled population growth or spread, it is possible that many perceived ‘lags’ in range expansion of human-associated species are caused by imperfect detection in natural environments. Small populations of invasive species can be difficult to detect and research is often biased towards invasive species that are already an obvious problem (Pysek et al. 2008).

Invasive populations, therefore, may not be detected in a new habitat until many years after establishment (Tingley et al. 2014).

Human-associated invasive species are likely to be more difficult to detect in complex natural environments than in anthropogenic habitats (Green et al. 2013). Furthermore, even if human-associated animals are detected in natural environments, they may be attributed to spillover from urban areas (i.e., immigration but no population growth), rather than established populations. It is, therefore, important to understand the factors that affect detection probability of human-associated species in order to develop effective methods for detecting individuals and to allow accurate estimates of abundance in natural environments.

The Asian house gecko, *Hemidactylus frenatus*

In this study I use the Asian house gecko, *Hemidactylus frenatus*, as a model system to investigate the processes associated with range expansion of a human-associated species into natural environments. Native to south and southeast Asia, this small gecko (Fig. 2) is a very successful invasive species, having been accidentally introduced to many tropical and subtropical areas around the world (Lever, 2003; Rödder et al., 2008; Hoskin, 2011). The introduced range of *H. frenatus* is currently known to include Australia, parts of South-East Asia, Japan, Mexico, the southern United States, Kenya, Madagascar, and many islands in the Indian, Pacific and Atlantic Oceans (Lever, 2003; Rödder et al., 2008; Hoskin, 2011). *Hemidactylus frenatus* has strong associations with humans and is commonly seen on buildings, hunting for insects around lights (Hoskin 2011, Zozaya et al. 2015). This species has been living in association with humans for so long that its natural habitat in its native range is largely unknown (Hoskin 2011).

Figure 2. An Asian house gecko (*Hemidactylus frenatus*) in an urban area. Photo: Stephen Zozaya.

Hemidactylus frenatus are highly efficient foragers and significantly reduce insect resources in urban areas, which often causes declines of native geckos (Hanley et al. 1998). Mark-recapture studies in the Pacific found that native geckos were 800% more common in urban habitats on islands where *H. frenatus* is absent than where it is present (Case et al. 1994). Although most studies of *H. frenatus* have taken place in urban areas, this species can also affect native geckos in natural habitats. On the Mascarene Islands, for example, *H. frenatus* competitively displace native *Nactus* geckos by excluding them from retreat sites, which has led to local extinctions of *Nactus* geckos in natural habitats (Cole et al. 2005). *Hemidactylus frenatus* are also known to introduce invasive parasites, although the impacts of these parasites on native geckos in the introduced range are largely unresolved (Hanley et al. 1995, 1998).

Despite the obvious impacts of *H. frenatus* and the fact that this species excels at human-mediated jump dispersal, generally not much concern is shown when *H. frenatus* is recorded in new places (Hoskin 2011). The reason for this is that most records of *H. frenatus* are from urban areas and it is commonly believed that, with the exception of islands, *H. frenatus* is restricted to highly-modified human environments (Vanderduys and Kutt 2013).

There are, however, a number of reasons why *H. frenatus* may be a greater threat to native geckos in natural environments than we currently acknowledge. First, *H. frenatus* establish large populations in urban areas (Case et al. 1994, Yang et al. 2012), which may result in many individuals entering surrounding natural environments. Second, *H. frenatus* excel at human-mediated jump dispersal (Chapple et al. 2016), and urban populations of *H. frenatus* are probably founded by individuals from multiple source regions, which could result in high levels of genetic diversity. Third, there have been an increasing number of *H. frenatus* records in natural environments (Hoskin 2011), and it is unclear whether these records reflect spillover from urban areas, recently introduced individuals (e.g., individuals recently transported on cars) or established populations. Fourth, due to their small size—with a maximum snout-vent length of approximately 60 mm (Hoskin 2011)—and cryptic nature, *H. frenatus* may be difficult to detect in natural environments, even if they are already out there, which could lead to perceived lags in range expansion.

In Australia, *H. frenatus* first established near Darwin in the 1960s (Fig. 3), and have since spread rapidly through human transport networks (Hoskin 2011). The current range of *H. frenatus* includes tropical and subtropical parts of the country (Fig. 3), and although this distribution is centred on urban areas, there have been an increasing number of *H. frenatus* records from natural habitats in Australia (Mckay et al., 2009; Hoskin, 2011).

Figure 3. The Australian distribution of *H. frenatus* taken from Hoskin (2011). Records were taken from museum specimen data, Hoskin's own records and the published literature. The arrow indicates the approximate location of the first established populations near Darwin in the Northern Territory and the orange rectangle shows the study region, Townsville in north Queensland.

It has been argued that *H. frenatus* can only thrive in highly modified anthropogenic environments in Australia (Vanderduys and Kutt 2013). This argument is based on data collected during broad ecological surveys in which most study sites were chosen to avoid human habitation and infrastructure (Vanderduys and Kutt 2013); however, for human-associated species, such as *H. frenatus,* the potential for range expansion into natural habitats needs to be investigated closer to the range edge (i.e., near the urban–natural interface). *Hemidactylus frenatus* has been recorded at more than one kilometre from the nearest structure on multiple occasions (Mckay et al. 2009, Hoskin 2011), which is likely much farther than an individual could disperse in a lifetime (Hoehn et al., 2007; Dubey et al., 2012; Paulissen et al., 2013). It is, however, unclear whether these individuals have been introduced through human mediated dispersal, or reflect established populations that have arrived in these areas by spreading through surrounding woodland.

Australia has high levels of gecko diversity and endemism, with over 160 species of gecko in 24 genera (Macdonald 2015), and it is critical to understand whether *H. frenatus* affect native geckos in natural environments here. *Hemidactylus frenatus* have introduced at least two species of novel parasite to Australia – the ectoparasitic mite, *Geckobia bataviensis* and the endoparasitic pentastomid, *Raillietiella frenata* (Hoskin 2011, Kelehear et al. 2014). It is currently unknown whether these parasites can be transmitted to native geckos in Australia, or whether *H. frenatus* compete with native geckos in natural habitats here.

The Study Region

Townsville is a city of approximately 180,000 people in north-east Australia (Figs 3 & 4; United Nations Statistics Division, 2016). *Hemidactylus frenatus* were first recorded in Townsville in 1980 (Hoskin 2011) and are now highly abundant in urban areas throughout the region (Pers. Obs.). Many urban areas in the Townsville region abut dry sclerophyll woodland (Fig. 4), which is habitat for a number of native gecko species (Macdonald 2015). In this region the urban–natural interface is large and varied, with urban areas of different sizes abutting different natural environments, ranging from high quality to degraded woodland (Fig. 4). This system provides an opportunity to assess whether *H. frenatus* can establish in natural environments and to investigate the factors that this facilitate range expansion across multiple semi-independent invasion fronts.

Figure 4. The Townsville region of north Queensland

Aims

In this thesis, I assess the potential for range expansion of *H. frenatus* into natural environments and investigate the factors that facilitate range expansion. Specifically, I aim to address the following questions:

1. Can *H. frenatus* establish viable populations in natural areas? And, if so, what demographic and environmental factors facilitate this range expansion?
2. Does morphological divergence occur during *H. frenatus* colonising natural environments?
3. Do parasites affect range expansion of *H. frenatus*? And could parasite transmission affect native geckos in natural environments?
4. How can we best detect *H. frenatus* in natural environments?

References

Aikio, S., Duncan, R.P., Hulme, P.E., 2010. Lag-phases in alien plant invasions: separating the facts from the artefacts. Oikos 119, 370–378. doi:10.1111/j.1600-0706.2009.17963.x

Andreou, D., Arkush, K.D., Guégan, J.-F., Gozlan, R.E., 2012. Introduced pathogens and native freshwater biodiversity: a case study of *Sphaerothecum destruens*. PLoS One 7, e36998. doi:10.1371/journal.pone.0036998

Boggs, C.L., Holdren, C.E., Kulahci, I.G., Bonebrake, T.C., Inouye, B.D., Fay, J.P., McMillan, A., Williams, E.H., Ehrlich, P.R., 2006. Delayed population explosion of an introduced butterfly. J. Anim. Ecol. 75, 466–475. doi:10.1111/j.1365-2656.2006.01067.x

Case, T.J., Bolger, D.T., Petren, K., 1994. Invasions and competitive displacement among house geckos in the tropical Pacific. Ecology 75, 464–477.

Case, T.J., Taper, M.L., 2000. Competition, environmental gradients, gene flow and the coevolution of species' borders. Am. Nat. 155, 583–605. doi:10.1086/303351

Chapple, D.G., Knegtmans, J., Kikillus, H., van Winkel, D., 2016. Biosecurity of exotic reptiles and amphibians in New Zealand building upon Tony Whitaker's legacy. J. R. Soc. New Zeal. 46, 1–9. doi:10.1080/02699931.2011.628301

Chapple, D.G., Simmonds, S.M., Wong, B.B.M., 2012. Can behavioral and personality traits influence the success of unintentional species introductions? Trends Ecol. Evol. 27, 57–64. doi:10.1016/j.tree.2011.09.010

Coates, A., Barnett, L.K., Hoskin, C., Phillips, B.L., In Press. Living on the edge: parasite prevalence changes dramatically across a range edge in an invasive gecko. Am. Nat.

Colautti, R.I., Grigorovich, I.A., MacIsaac, H.J., 2006. Propagule pressure: a null model for biological invasions. Biol. Invasions 8, 1023–1037. doi:10.1007/s10530-005-3735-y

Cole, N.C., Jones, C.G., Harris, S., 2005. The need for enemy-free space: the impact of an invasive gecko on island endemics. Biol. Conserv. 125, 467–474. doi:10.1016/j.biocon.2005.04.017

Colla, S.R., Otterstatter, M.C., Gegear, R.J., Thomson, J.D., 2006. Plight of the bumble bee: pathogen spillover from commercial to wild populations. Biol. Conserv. 129, 461–467. doi:10.1016/j.biocon.2005.11.013

Crooks, J.A., 2005. Lag times and exotic species: the ecology and management of biological invasions in slow-motion. Ecoscience 12, 316–329. doi:10.2980/i1195-6860-12-3-316.1

Dubey, S., Croak, B., Pike, D., Webb, J., Shine, R., 2012. Phylogeography and dispersal in the velvet gecko (*Oedura lesueurii*), and potential implications for conservation of an endangered snake (*Hoplocephalus bungaroides*). BMC Evol. Biol. 12, 67. doi:10.1186/1471-2148-12-67

Dunn, A.M., Hatcher, M.J., 2015. Parasites and biological invasions: parallels, interactions, and control. Trends Parasitol. 31, 189–199. doi:10.1016/j.pt.2014.12.003

Gaither, M.R., Toonen, R.J., Bowen, B.W., 2012. Coming out of the starting blocks: extended lag time rearranges genetic diversity in introduced marine fishes of Hawai‘i. Proc. R. Soc. B Biol. Sci. 279, 3948–3957. doi:10.1098/rspb.2012.1481

Gomulkiewicz, R., Holt, R.D., Barfield, M., 1999. The effects of density dependence and immigration on local adaptation and niche evolution in a black-hole sink environment. Theor. Popul. Biol. 55, 283–96. doi:10.1006/tpbi.1998.1405

Green, S.J., Tamburello, N., Miller, S.E., Akins, J.L., Cote, I.M., 2013. Habitat complexity and fish size affect the detection of Indo-Pacific lionfish on invaded coral reefs. Coral Reefs 32, 413–421. doi:10.1007/s00338-012-0987-8

Grimm, N.B., Faeth, S.H., Golubiewski, N.E., Redman, C.L., Wu, J., Bai, X., Briggs, J.M., 2008. Global change and the ecology of cities. Science. 319, 756–760. doi:10.1126/science.1150195

Hanley, K.A., Fisher, R.N., Case, T.J., 1995. Lower mite infestations in an asexual gecko compared with its sexual ancestors. Evolution. 49, 418–426.

Hanley, K.A., Petren, K., Case, T.J., 1998. An experimental investigation of the competitive displacement of a native gecko by an invading gecko: no role for parasites. Oecologia 115, 196–205. doi:10.1007/s004420050508

Higham, T.E., Russell, A.P., 2010. Divergence in locomotor performance, ecology, and morphology between two sympatric sister species of desert-dwelling gecko. Biol. J. Linn. Soc. 101, 860–869. doi:10.1111/j.1095-8312.2010.01539.x

Hoehn, M., Sarre, S.D., Henle, K., 2007. The tales of two geckos: does dispersal prevent extinction in recently fragmented populations? Mol. Ecol. 16, 3299–3312. doi:10.1111/j.1365-294X.2007.03352.x

Holt, R.D., Knight, T.M., Barfield, M., 2004. Allee effects, immigration, and the evolution of species' niches. Am. Nat. 163, 253–262. doi:10.1086/381408

Hoskin, C.J., 2011. The invasion and potential impact of the Asian House Gecko (*Hemidactylus frenatus*) in Australia. Austral Ecol. 36, 240–251. doi:10.1111/j.1442-9993.2010.02143.x

Hulme, P.E., 2009. Trade, transport and trouble: managing invasive species pathways in an era of globalization. J. Appl. Ecol. 46, 10–18. doi:10.1111/j.1365-2664.2008.01600.x

Irschick, D.J., Garland, T., 2001. Integrating function and ecology in studies of adaptation: investigations of locomotor capacity as a model system. Annu. Rev. Ecol. Syst. 32, 367–396.

Jeschke, J.M., Strayer, D.L., 2006. Determinants of vertebrate invasion success in Europe and North America. Glob. Chang. Biol. 12, 1608–1619. doi:10.1111/j.1365-2486.2006.01213.x

Kelehear, C., Saltonstall, K., Torchin, M.E., 2014. An introduced pentastomid parasite (*Raillietiella frenata*) infects native cane toads (*Rhinella marina*) in Panama. Parasitology 142, 675–679. doi:10.1017/S0031182014001759

Keller, A., 2007. *Drosophila melanogaster*' s history as a human commensal. Curr. Biol. 17, 77–81.

Kelly, D.W., Paterson, R.A., Townsend, C.R., Poulin, R., Tompkins, D.M., 2009. Parasite spillback: a neglected concept in invasion ecology. Ecology 90, 2047–2056. doi:10.1177/03063127067078012

Kotler, B.P., Brown, J.S., Smith, R.J., Wirtz II, W.O., 1988. The effects of morphology and body size on rates of owl predation on desert rodents. Oikos 53, 145–152.

Lee, C.E., 2002. Evolutionary genetics of invasive species. Trends Ecol. Evol. 17, 386–391. doi:http://dx.doi.org/10.1016/S0169-5347(02)02554-5

Leger, E.A., Rice, K.J., 2007. Assessing the speed and predictability of local adaptation in invasive California poppies (*Eschscholzia californica*). J. Evol. Biol. 20, 1090–1103. doi:10.1111/j.1420-9101.2006.01292.x

Lei, J., Booth, D.T., 2014. Temperature, field activity and post-feeding metabolic response in the Asian house gecko, *Hemidactylus frenatus*. J. Therm. Biol. 45, 175–80. doi:10.1016/j.jtherbio.2014.09.006

Lever, C., 2003. Naturalized reptiles and amphibians of the world. Oxford University Press, New York.

Lockwood, J.L., Cassey, P., Blackburn, T., 2005. The role of propagule pressure in explaining species invasions. Trends Ecol. Evol. 20, 223–8. doi:10.1016/j.tree.2005.02.004

Losos, J.B., Warheit, K.I., Schoener, T.W., 1997. Adaptive differentiation following experimental island colonization in *Anolis* lizards. Nature 387, 70–73. doi:10.1038/387070a0

Lymbery, A.J., Morine, M., Kanani, H.G., Beatty, S.J., Morgan, D.L., 2014. Co-invaders: The effects of alien parasites on native hosts. Int. J. Parasitol. Parasites Wildl. 3, 171–177. doi:10.1016/j.ijppaw.2014.04.002

Macdonald, S., 2015. Australian Reptiles Online Database [WWW Document]. URL http://www.arod.com.au/arod/reptilia/Squamata/Gekkonidae/

Mack, R.N., Simberloff, D., Lonsdale, M.W., Evans, H., Clout, M., Bazzaz, F.A., 2000. Biotic invasions: causes, epidemiology, global consequences and control. Ecol. Appl. 10, 689–710. doi:10.1890/1051-0761(2000)010[0689:bicegc]2.0.co;2

Martin, L.J., Adams, R.I., Bateman, A., Bik, H.M., Hawks, J., Hird, S.M., Hughes, D., Kembel, S.W., Kinney, K., Kolokotronis, S.-O., Levy, G., McClain, C., Meadow, J.F., Medina, R.F., Mhuireach, G., Moreau, C.S., Munshi-South, J., Nichols, L.M., Palmer, C., Popova, L., Schal, C., Täubel, M., Trautwein, M., Ugalde, J. a., Dunn, R.R., 2015. Evolution of the indoor biome. Trends Ecol. Evol. 30, 223–232. doi:10.1016/j.tree.2015.02.001

Mckay, J.L., Griffiths, A.D., Crase, B., 2009. Distribution and habitat use by *Hemidactylus frenatus* Dumeril and Bibron (Gekkonidae) in the Northern Territory. Beagle Rec. Museums Art Gall. North. Territ. 25, 107–112.

Møller, A.P., 2008. Flight distance of urban birds, predation, and selection for urban life. Behav. Ecol. Sociobiol. 63, 63–75. doi:10.1007/s00265-008-0636-y

Mukherjee, A., Williams, D.A., Wheeler, G.S., Cuda, J.P., Pal, S., Overholt, W.A., 2011. Brazilian peppertree (*Schinus terebinthifolius*) in Florida and South America: evidence of a possible niche shift driven by hybridization. Biol. Invasions 14, 1415–1430. doi:10.1007/s10530-011-0168-7

O’Dowd, D.J., Green, P.T., Lake, P.S., 2003. Invasional “meltdown” on an oceanic island. Ecol. Lett. 6, 812–817. doi:10.1046/j.1461-0248.2003.00512.x

Paulissen, M.A., Meyer, H.A., Hibbs, T.S., 2013. Movement patterns and sociality of the Mediterranean gecko, *Hemidactylus turcicus*, in Southwestern Louisiana. Southwest. Nat. 58, 344–350. doi:10.3398/064.074.0118

Petren, K., Case, T.J., 1998. Habitat structure determines competition intensity and invasion success in gecko lizards. Proc. Natl. Acad. Sci. U. S. A. 95, 11739–11744. doi:10.1073/pnas.95.20.11739

Phillips, B.L., Brown, G.P., Greenlees, M., Webb, J.K., Shine, R., 2007. Rapid expansion of the cane toad (*Bufo marinus*) invasion front in tropical Australia. Austral Ecol. 32, 169–176. doi:10.1111/j.1442-9993.2007.01664.x

Phillips, B.L., Kelehear, C., Pizzatto, L., Brown, G.P., Barton, D., Shine, R., 2010. Parasites and pathogens lag behind their host during periods of host range advance. Ecology 91, 872–81.

Prentis, P.J., Wilson, J.R.U., Dormontt, E.E., Richardson, D.M., Lowe, A.J., 2008. Adaptive evolution in invasive species. Trends Plant Sci. 13, 288–294. doi:http://dx.doi.org/10.1016/j.tplants.2008.03.004

Pysek, P., Richardson, D.M., Pergl, J., Jarosik, V., Sixtova, Z., Weber, E., 2008. Geographical and taxonomic biases in invasion ecology. Trends Ecol. Evol. 23, 237–244. doi:10.1016/j.tree.2008.02.002

Randa, L.A., Yunger, J.A., 2006. Carnivore occurrence along an urban-rural gradient:

A landscape-level analysis. J. Mammal. 87, 1154–1164.

Richardson, D.M., Pysek, P., Simberloff, D., Rejmanek, M., Mader, A.D., 2008. Biological invasions - the widening debate: a response to Charles Warren. Prog. Hum. Geogr. 32, 295–298. doi:10.1177/0309132507088313

Richardson, D.M., Ricciardi, A., 2013. Misleading criticisms of invasion science: a field guide. Divers. Distrib. 19, 1461–1467. doi:10.1111/ddi.12150

Rilov, G., Benayahu, Y., Gasith, A., 2004. Prolonged lag in population outbreak of an invasive mussel: a shifting-habitat model. Biol. Invasions 6, 347–364. doi:10.1023/b:binv.0000034614.07427.96

Rödder, D., Solé, M., Böhme, W., 2008. Predicting the potential distribution of two alien invasive housegeckos (Gekkonidae: *Hemidactylus frenatus, Hemidactylus mabouia*). North. West. J. Zool. 4, 236–246.

Sætre, G.P., Riyahi, S., Aliabadian, M., Hermansen, J.S., Hogner, S., Olsson, U., Gonzalez Rojas, M.F., Sæther, S.A., Trier, C.N., Elgvin, T.O., 2012. Single origin of human commensalism in the house sparrow. J. Evol. Biol. 25, 788–796. doi:10.1111/j.1420-9101.2012.02470.x

Savard, J.-P.L., Clergeau, P., Mennechez, G., 2000. Biodiversity concepts and urban ecosystems. Landsc. Urban Plan. 48, 131–142. doi:DOI: 10.1016/S0169-2046(00)00037-2

Short, K.H., Petren, K., 2012. Rapid species displacement during the invasion of Florida by the tropical house gecko *Hemidactylus mabouia*. Biol. Invasions 14, 1177–1186. doi:10.1007/s10530-011-0147-z

Simberloff, D., 2009. The role of propagule pressure in biological invasions. Annu. Rev. Ecol. Evol. Syst. 40, 81–102. doi:doi:10.1146/annurev.ecolsys.110308.120304

Simberloff, D., Nuñez, M.A., Ledgard, N.J., Pauchard, A., Richardson, D.M., Sarasola, M., Van Wilgen, B.W., Zalba, S.M., Zenni, R.D., Bustamante, R., Peña, E., Ziller, S.R., 2010. Spread and impact of introduced conifers in South America: Lessons from other southern hemisphere regions. Austral Ecol. 35, 489–504. doi:10.1111/j.1442-9993.2009.02058.x

Suarez, A. V, Tsutsui, N.D., 2008. The evolutionary consequences of biological invasions. Mol. Ecol. 17, 351–60. doi:10.1111/j.1365-294X.2007.03456.x

Svenning, J.-C., Gravel, D., Holt, R.D., Schurr, F.M., Thuiller, W., Münkemüller, T., Schiffers, K.H., Dullinger, S., Edwards, T.C., Hickler, T., Higgins, S.I., Nabel, J.E.M.S., Pagel, J., Normand, S., 2014. The influence of interspecific interactions on species range expansion rates. Ecography (Cop.). 37, 1–12. doi:10.1111/j.1600-0587.2013.00574.x

Tingley, R., Weeks, A.R., Smart, A.S., van Rooyen, A.R., Woolnough, A.P., McCarthy, M.A., 2014. European newts establish in Australia, marking the arrival of a new amphibian order. Biol. Invasions. doi:10.1007/s10530-014-0716-z

United Nations Statistics Division, T., 2016. UNdata [WWW Document]. UNSD Demogr. Statisitics. URL http://data.un.org/Data.aspx?d=POP&f=tableCode%3A240#POP

Urban, M.C., Phillips, B.L., Skelly, D.K., Shine, R., 2007. The cane toad's (*Chaunus [Bufo] marinus*) increasing ability to invade Australia is revealed by a dynamically updated range model. Proc. R. Soc. B Biol. Sci. 274, 1413–1419. doi:10.1098/rspb.2007.0114

Vanderduys, E.P., Kutt, A.S., 2013. Is the Asian house gecko, *Hemidactylus frenatus*, really a threat to Australia's biodiversity? Aust. J. Zool. 60, 361–367.

Vervust, B., Grbac, I., Van Damme, R., 2007. Differences in morphology, performance and behaviour between recently diverged populations of *Podarcis sicula* mirror differences in predation pressure. Oikos 116, 1343–1352. doi:10.1111/j.0030-1299.2007.15989.x

Wanless, R.M., Angel, A., Cuthbert, R.J., Hilton, G.M., Ryan, P.G., 2007. Can predation by invasive mice drive seabird extinctions? Biol. Lett. 3, 241–244. doi:10.1098/rsbl.2007.0120

Wikelski, M., 2005. Evolution of body size in Galapagos marine iguanas. Proc. R. Soc. B Biol. Sci. 272, 1985–1993. doi:10.1098/rspb.2005.3205

Wilson, J.R.U., Dormontt, E.E., Prentis, P.J., Lowe, A.J., Richardson, D.M., 2009. Something in the way you move: dispersal pathways affect invasion success. Trends Ecol. Evol. 24, 136–144. doi:http://dx.doi.org/10.1016/j.tree.2008.10.007

Winchell, K.M., Reynolds, R.G., Prado-Irwin, S.R., Puente-Rolón, A.R., Revell, L.J., 2016. Phenotypic shifts in urban areas in the tropical lizard *Anolis cristatellus*. Evolution (N. Y). 70, 1009–1022. doi:10.1111/evo.12925

Yang, D., González-Bernal, E., Greenlees, M., Shine, R., 2012. Interactions between native and invasive gecko lizards in tropical Australia. Austral Ecol. 37, 592–599. doi:10.1111/j.1442-9993.2011.02319.x

Zozaya, S.M., Alford, R.A., Schwarzkopf, L., 2015. Invasive house geckos are more willing to use artificial lights than are native geckos. Austral Ecol. 40, 982–987. doi:10.1111/aec.12287

Zuk, M., Rotenberry, J.T., Tinghitella, R.M., 2006. Silent night: adaptive disappearance of a sexual signal in a parasitized population of field crickets. Biol. Lett. 2, 521–4. doi:10.1098/rsbl.2006.0539

Chapter 2.

Going feral: Time and propagule pressure determine range expansion of Asian house geckos into natural environments

Louise K Barnett[1], Ben L Phillips[2] & Conrad J Hoskin[1]

[1]*Centre for Tropical Biodiversity & Climate Change, College of Science & Engineering, James Cook University, Townsville, Queensland 4811, Australia.*
[2]*School of Biosciences, University of Melbourne, Parkville, Victoria 3010, Australia.*

Manuscript Published in: *Austral Ecology*

Austral
ECOLOGY A Journal of ecology in the Southern Hemisphere

Austral Ecology (2017) **42**, 165–175

Going feral: Time and propagule pressure determine range expansion of Asian house geckos into natural environments

LOUISE K. BARNETT,[1]* BEN L. PHILLIPS[2] AND CONRAD J. HOSKIN[1]
[1]College of Marine & Environmental Sciences, James Cook University, Townsville, Queensland 4811 (E-mail: louisekbarnett@gmail.com), and [2]School of Biosciences, University of Melbourne, Parkville, Victoria, Australia

Abstract Upon establishment in a new area, invasive species may undergo a prolonged period of relatively slow population growth and spread, known as a lag period. Lag periods are, apparently, common in invasions, but studies of the factors that facilitate subsequent expansions are lacking in natural systems. We used 10 semi-independent invasions of the Asian house gecko (*Hemidactylus frenatus*) to investigate which factors facilitate expansion of this human-associated species across the urban–woodland interface. We conducted 590 surveys over 12 months on 10 transects running from the urban edge to 2 km into adjacent natural woodland. We recorded *H. frenatus* out to 2 km from the urban edge on nine of 10 transects, and at high abundance at many woodland sites. Body size, body condition, sex ratio and proportion of gravid females did not vary with distance from the urban edge, suggesting viable, self-sustaining populations in natural habitats. The extent of expansion was, however, strongly dependent on propagule pressure (the abundance of *H. frenatus* at the urban edge), and time (time since *H. frenatus* established in the urban area). The size of the urban area and the structure of the surrounding environment did not impact invasion. Our results show that an invasive species that is deemed 'human-associated' over most of its range is invading natural habitats, and propagule pressure strongly controls the lag time in this system, a finding that echoes results for establishment probability at larger scales.

Key words: *Hemidactylus frenatus*, invasive species, lag period, range expansion, urbanization.

doi:10.1111/aec.12416

Chapter 2

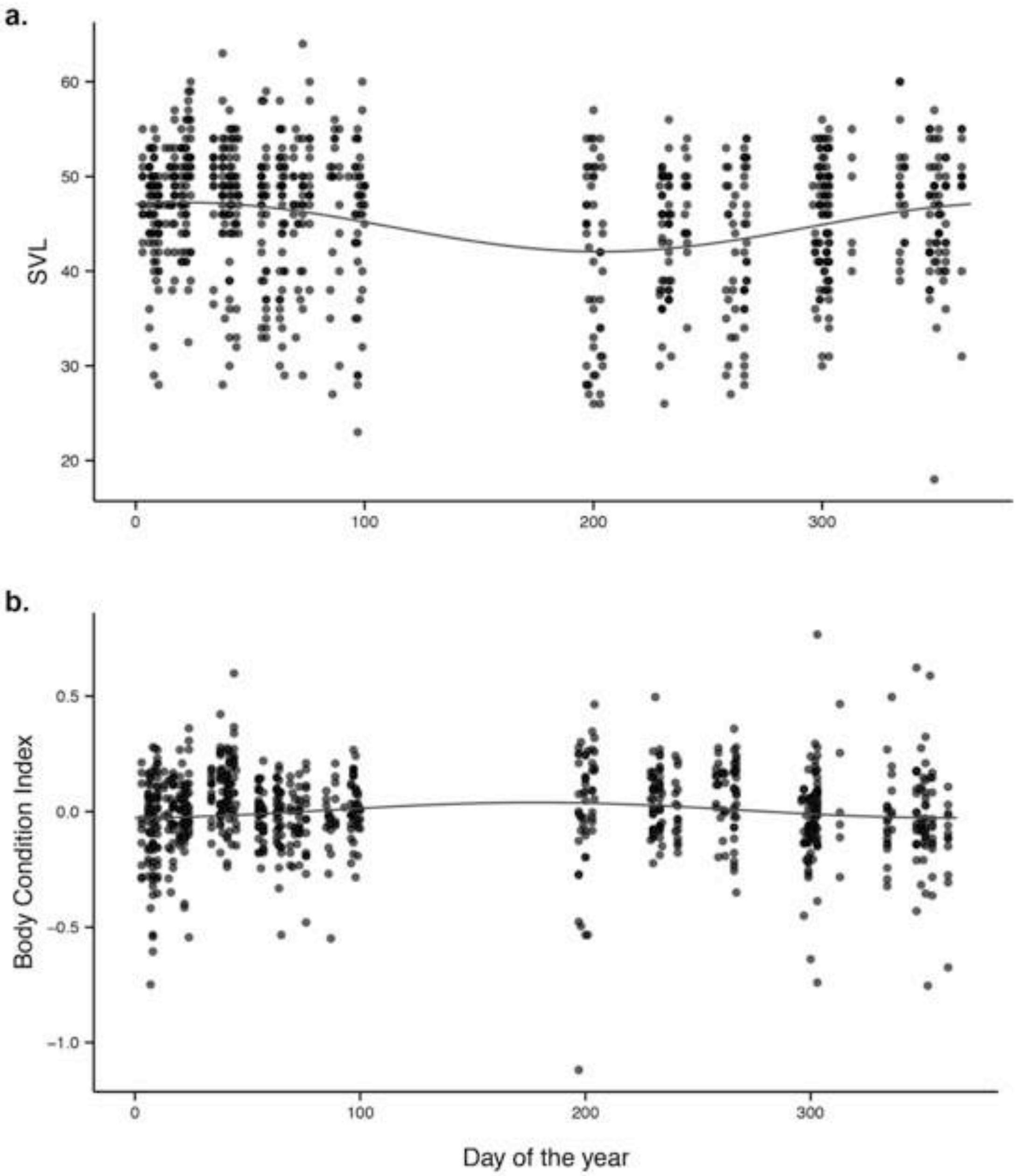
Supplementary Material

Supplementary Table 1. Model comparison of the distance and habitat models using Akaike Information Criteria (AIC). A log link function was used for the following GLMER models.

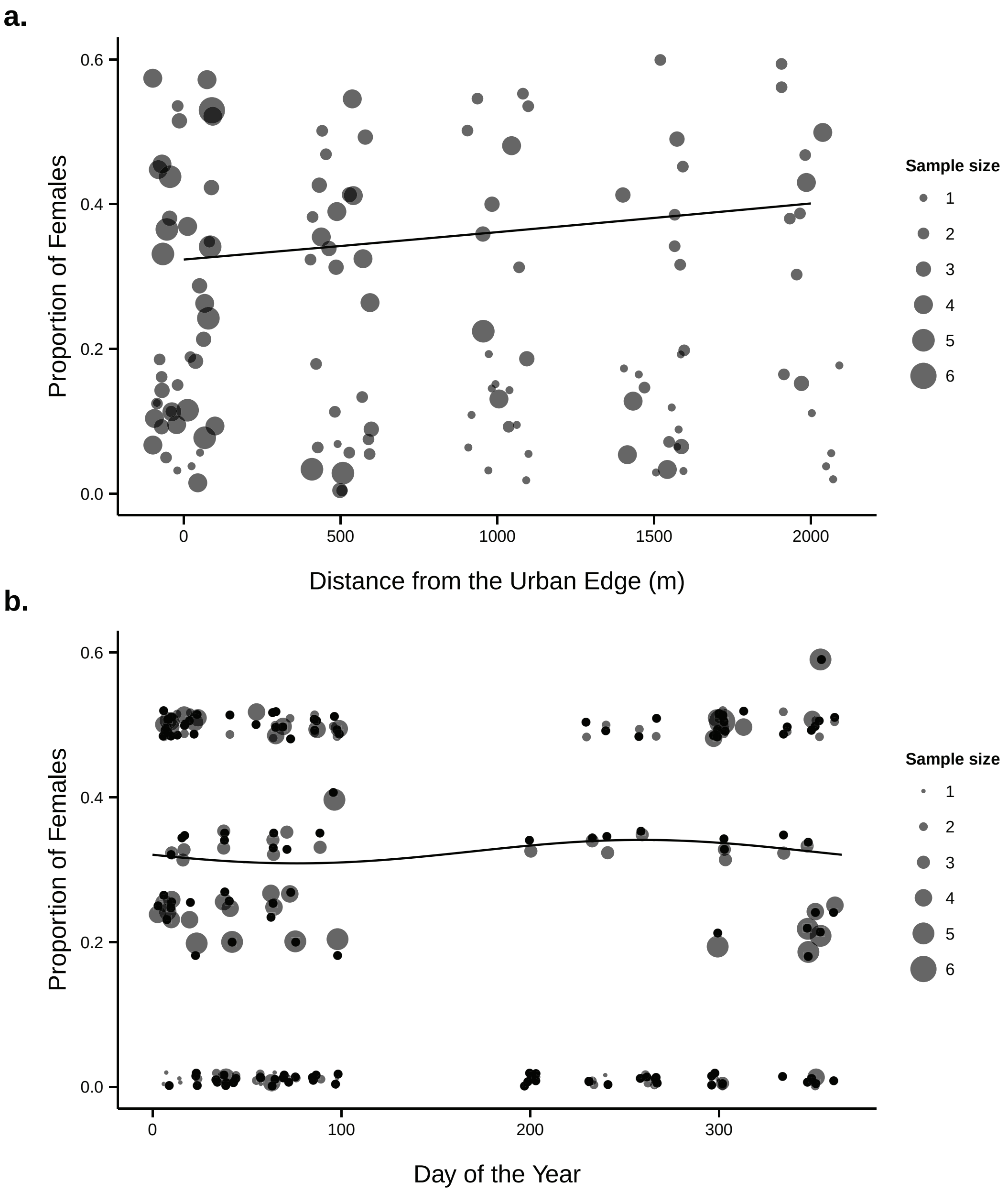
Model	**Type**	**df**	**AIC**
Prop. Females ~ Season + Distance	GLMER (binomial)	5	484.18
Prop. Females ~ Season + Habitat	GLMER (binomial)	5	485.18
Prop. Gravid Females ~ Season + Distance	GLMER (binomial)	5	317.48
Prop. Gravid Females ~ Season + Habitat	GLMER (binomial)	5	316.96
Prop. Juveniles ~ Season + Distance	GLMER (binomial)	5	692.51
Prop Juveniles ~ Season + Habitat	GLMER (binomial)	5	687.42
SVL ~ Season + Distance	LMER	6	5696.14
SVL ~ Season + Habitat	LMER	6	5695.39
BCI ~ Season + Distance	LMER	6	-490.59
BCI ~ Season + Habitat	LMER	6	-490.40

Supplementary Table 2. Results from the habitat and distance models evaluating whether *H. frenatus* populations in natural environments are self-sustaining.

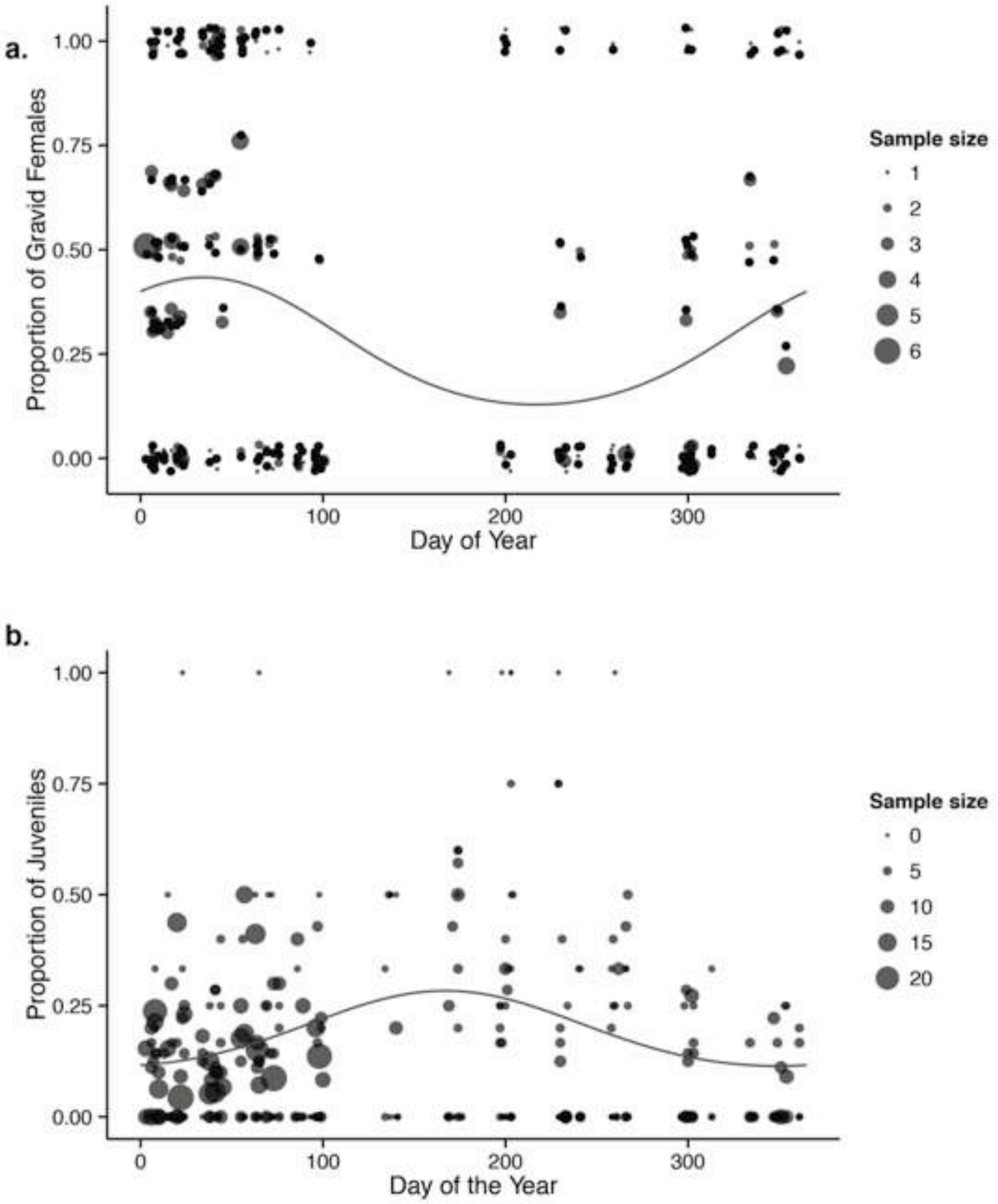
	Distance / Location				**Sine(day)**				**Cosine(day)**			
Model	Estimate	Std error	T/ Z	P	Estimate	Std error	T/Z	P	Estimate	Std error	T/Z	P
Prop. Females ~ Distance + Season	0.11	0.13	-4.55	< 0.001	-0.05	0.09	-0.53	0.59	-0.01	0.12	-0.12	0.91
Prop. Females ~ Habitat + Season	0.17	0.19	-0.92	0.36	-0.05	0.09	-0.59	0.55	-0.02	0.12	-0.18	0.85
Prop. Gravid Females ~ Distance + Season	0.16	0.11	1.42	0.16	0.31	0.13	2.36	0.02	0.49	0.18	2.70	< 0.01
Prop. Gravid Females ~ Habitat + Season	-0.40	0.26	-1.58	0.11	0.31	0.13	2.32	0.02	0.48	0.18	2.69	< 0.01
Prop. Juveniles ~ Distance + Season	-0.278	0.074	-3.790	< 0.001	0.100	0.072	1.407	0.159	-0.391	0.073	-5.382	< 0.001
Prop. Juveniles ~ Habitat + Season	0.61	0.14	4.50	< 0.001	0.00	0.07	1.35	0.18	-0.39	0.07	-5.34	< 0.001
SVL ~ Distance + Season	0.24	0.23	1.03	0.30	0.61	0.24	2.58	0.01	1.66	0.28	6.03	< 0.001
SVL ~ Habitat + Season	-0.29	0.47	-0.61	0.54	0.60	0.23	2.54	0.01	1.66	0.28	6.05	<0.001
BCI ~ Distance + Season	0.01	0.01	1.28	0.20	0.00	0.01	0.29	0.77	-0.02	0.01	-3.09	< 0.01
BCI ~ Habitat + Season	0.00	0.01	-0.08	0.93	0.00	0.01	0.22	0.83	-0.02	0.01	-3.04	< 0.01



Supplementary Figure 1. Seasonal effects on **(a)** *H. frenatus* body size (snout-vent length) and **(b)** body condition.



Supplementary Figure 2. The sex ratio of *H. frenatus* captured per survey with **(a)** distance from the urban edge, and **(b)** season (day of year).



Supplementary Figure 3. Seasonal variation in **(a)** the proportion of gravid *H. frenatus*, and **(b)** the proportion of juvenile *H. frenatus* found.

Supplementary Figure 4. Examples of the habitat studied at: **(a)** the urban edge site and **(b)** an adjacent woodland site on the Town Common Conservation Park transect. This figure illustrates that urban environments are more structurally complex than surrounding natural habitats (with a greater number of potential refuge and foraging sites for arboreal geckos). Urban areas also have artificial lights (and associated insect resources). These two differences likely result in an increased carrying capacity for *H. frenatus* in urban environments compared to surrounding woodland.

Chapter 3.

Morphological divergence in woodland populations of a human-associated invasive gecko

Louise K Barnett[1], Megan Higgie[1], Ben L Phillips[2] & Conrad J Hoskin[1]

[1]*Centre for Tropical Biodiversity & Climate Change, College of Science & Engineering, James Cook University, Townsville, Queensland 4811, Australia.*
[2]*School of Biosciences, University of Melbourne, Parkville, Victoria 3010, Australia.*

17 March 2017

Manuscript for consideration in: *Biological Invasions*

Abstract

Invasive species often undergo phenotypic changes, which may facilitate establishment or range expansion in novel environments. These behavioural, morphological or physiological changes may arise through either adaptations or plasticity. While human-associated invasive species thrive in anthropogenic environments, phenotypic divergence may be important during subsequent expansion into natural habitats. The Asian house gecko, *Hemidactylus frenatus*, is a globally widespread invasive species with strong ties to humans and their infrastructure. Large populations of *H. frenatus* have recently been recorded in natural environments, providing an ideal system to assess whether morphological divergence occurs during range expansion of a human-associated species. We assessed differences in body condition, body size and shape, and toe size and shape, between *H. frenatus* populations in urban and natural habitats across semi-independent invasion fronts around a city in north-east Australia. Males were significantly smaller in woodland environments than urban areas, but female size was similar between urban and woodland habitats. Male body shape was similar in urban populations, but body shape differed for males in different woodland populations. Toe shape results were complex, with differences in toe shape detected between urban and woodland populations at one invasion front but not at the others. Body condition also differed between the invasion fronts. Our results indicate that *H. frenatus* have morphologically diverged in natural environments, but in ways that differ between the semi-independent invasion fronts studied.

Key words: range expansion; invasive; divergence; morphology; *Hemidactylus frenatus*

Introduction

Invasive species are a major conservation concern, and globalisation has led to an increase in species introductions (Hulme 2009, Richardson and Ricciardi 2013). Although they are a conservation issue, invasive species also provide interesting evolutionary systems. These species are introduced to environments with novel predators, competitors, parasites, climates and habitats. Establishment and spread in these novel environments is often associated with changes in phenotype (Rejmánek and Richardson 1996, Holway and Suarez 1999, Prentis et al. 2008, Davidson et al. 2011).

Phenotypic changes in invasive species may arise through adaptive evolution or phenotypic plasticity, both of which can result in divergence over relatively short time-scales (Urban et al. 2007, Kolbe et al. 2012). Furthermore, these adaptive or plastic shifts may contribute to invasion success in novel habitats (Ghalambor et al. 2007, Davidson et al. 2011). While genetic analyses or common garden experiments are necessary to understand the basis of phenotypic divergence (Winchell et al. 2016), the first step is to assess whether trait changes are associated with colonisation of a novel habitat.

Human-associated invasive species thrive in anthropogenic habitats and often establish quickly upon introduction to urban areas (Sætre et al. 2012). Phenotypic divergence, however, may be important during subsequent expansion into natural environments. Natural habitats differ from urban areas in many important ways, including structural complexity, physical surfaces, predation pressure, food availability, thermal regime and light environment (Jokimäki and Huhta 2000, Mörtberg and Wallentinus 2000, Alberti et al. 2003, Møller 2008). These environmental differences may select for shifts in morphological, behavioural or physiological traits. Recently, for example, Winchell et al. (2016) documented morphological adaptations in the lizard *Anolis cristatellus* upon colonisation of urban areas; but little research has investigated the reverse: whether phenotypic divergence occurs during expansion of human-associated species into natural environments (Mukherjee et al. 2011).

For species transitioning abrupt habitat boundaries like the urban–natural interface, morphological divergence is likely. Morphological traits govern how organisms interact with their environment, affecting susceptibility to predation (Kotler et al. 1988, Vervust et al. 2007), thermal tolerance (Wikelski 2005), foraging efficiency and locomotor performance (Irschick and Garland 2001, Higham and Russell 2010); and optimal morphological traits likely differ between urban and natural environments. For example, *Anolis* lizards in urban environments have more sub-digital lamellae and longer limbs compared to those in natural environments, and this trait likely helps them to grip on the broad surfaces in urban areas (Winchell et al. 2016). Urban areas are also likely to harbour larger populations of certain species (Bolger et al. 1997, Shochat et al. 2010), which may result in more intense competition and a competitive advantage for larger bodied individuals in this habitat. As such, one might expect populations colonising urban habitats to attain larger body sizes than their woodland counterparts. Such predictions can, however, be complicated by plastic responses. For example, large body sizes might be an advantage under intermediate levels of competition in urban environments, however, if competition is too high, small-bodied individuals might have an advantage.

It is clear that habitat differences between urban and natural environments may result in individual trait shifts (e.g., larger toe pads in urban lizard populations; Winchell et al. 2016). However, the direction of these changes can be strongly affected by plasticity and it may be difficult to interpret the ecological significance of such divergence. There is, nonetheless, a broader contrast in variability between the two habitats, and while urban areas tend to be quite similar in different regions (e.g., all have flat surfaces, artificial lights, similar predation regimes etc.), natural environments are likely to be much more variable across regions, with different habitat structures and species assemblages (Losos et al. 1997, Shochat et al. 2010). Therefore, while colonisation of different urban habitats may often involve convergent trait shifts (e.g., Winchell et al. 2016), colonisation of different natural environments may involve divergent trait shifts (e.g., Losos et al. 1997).

The Asian house gecko, *Hemidactylus frenatus*

Asian house geckos *(Hemidactylus frenatus)* are highly adapted to anthropogenic environments, and close associations with humans have facilitated their accidental introduction to many subtropical and tropical areas around the world (Lever 2003, Rödder et al. 2008, Hoskin 2011). They now have a pantropical distribution centred around urban areas and have been associated with humans for so long that even in their native range of south and Southeast Asia, their ancestral natural habitat is largely unknown (Hoskin 2011).

Hemidactylus frenatus thrive in urban areas for a number of reasons. First, as small animals with a mean snout–vent length of between 49.5 mm and 52.5 mm (Yang et al. 2012; Barnett et al. 2017), they can shelter easily in crevices on buildings. Second, as well as claws, *H. frenatus* have adhesive toepads (sub-digital lamellae) that allow them to navigate very smooth surfaces, such as window glass, in urban environments (Cole et al. 2005). Third, *H. frenatus* are behaviourally flexible and hunt for insects around artificial lights (Hanley et al. 1996, Zozaya et al. 2015). Such ‘clumping’ of insect resources appears to increase the competitive ability of *H. frenatus* (Petren and Case 1998), and in much of their introduced range they displace native geckos (e.g., *Lepidodactylus lugubris*) in urban environments (Case and Bolger 1991, Hanley et al. 1996). Buildings also provide thermal buffering for *H. frenatus*, allowing them to persist in urban areas during parts of the year that may otherwise be too cold (Lei and Booth 2014).

Despite being so well suited to urban life, in northern Australia *H. frenatus* can also thrive in some natural environments (Barnett et al. 2017). In Australia, *H. frenatus* first established in the 1960s, but self-sustaining populations in natural habitats have only been demonstrated recently (Barnett et al. 2017). We know that range expansion of *H. frenatus* into natural environments is determined by propagule pressure (i.e., the abundance of *H. frenatus* in the adjacent urban area) and time since establishment in the urban area (Barnett et al. 2017); however, it is unclear whether morphological change also facilitates expansion into natural environments.

Here, we address whether *H. frenatus* populations in natural woodland environments differ from urban populations in body or toe morphology. We predicted that urban geckos would have larger toe pads and more sub-digital lamellae than their woodland counterparts (because urban areas have proportionally more smooth surfaces), and that urban geckos would be larger bodied due increased competition in urban environments.

Our overall goal was to use data collected from multiple urban–woodland invasion fronts to test whether: 1. woodland populations all show parallel morphological trait shifts from urban populations, or 2. woodland populations show more variation in traits across sites than urban populations because woodland environments are more variable than urban environments. To address these Aims we use two different data sets: field-measured body size data collected from 577 wild geckos from 10 largely independent invasion fronts; and lab-measured body and toe size and shape data collected from 119 wild geckos from three independent invasion fronts.

Methods

Field-measured Body Size

We used data collected during a previous study of 10 urban–woodland transects in the Townsville region (Barnett et al. 2017) to assess whether snout-vent length (SVL) varied between urban and woodland populations of *H. frenatus* (Fig. 1). This effort provided a very large dataset (N = 577 individuals) across 10 semi-independent invasion fronts surrounding Townsville city. These data were used to assess gecko body size in different habitats and locations.

We collected body size from *H. frenatus* at the 10 transects over a period of eight months (Fig. 1). Each transect was made up of five sites: the first site was amongst houses on the urban edge, and the other four sites were positioned every 500 m out to 2 km into adjacent woodland (Supp. Fig. 1). Transects locations were: the Australian Institute of Marine Science (AI), the Town Common Conservation Park (TC), Magnetic

Island (MI), Toomulla (TO), Toolakea (TK), Bluewater Park (BP), Yabulu (YA), Cungulla (CU), Mount Elliott (ME), and Mount Stuart (MS) (Fig. 1). All 10 transects were located within 50 km of Townsville city. We surveyed each transect once a month from August 2013 to April 2014. Head torches were used to locate *H. frenatus* by their eye-shine, and geckos were captured by hand. Up to five *H. frenatus* were measured per survey at each site.

We measured snout-vent length (SVL) in the wild using a transparent plastic ruler. Sex was determined by visually checking for the enlarged testes bulges of males and tail condition was recorded as original or regenerated. The age class was recorded as juvenile, sub adult, or adult, based on visual assessment of each gecko. Only measurements from adult *H. frenatus* were used for this study. After being measured, *H. frenatus* were released at the point of capture.

We measured a total of 296 adult male and 281 adult female *H. frenatus* throughout these surveys and geckos were grouped into two habitat categories, ‘urban’ and ‘woodland’, depending on where they were found. *Hemidactylus frenatus* found at the urban edge (Site 1) were placed in the ‘urban’ habitat category, while those found between 500 m and 2 km from the urban edge (Sites 2–5) were placed in the ‘woodland’ habitat category.

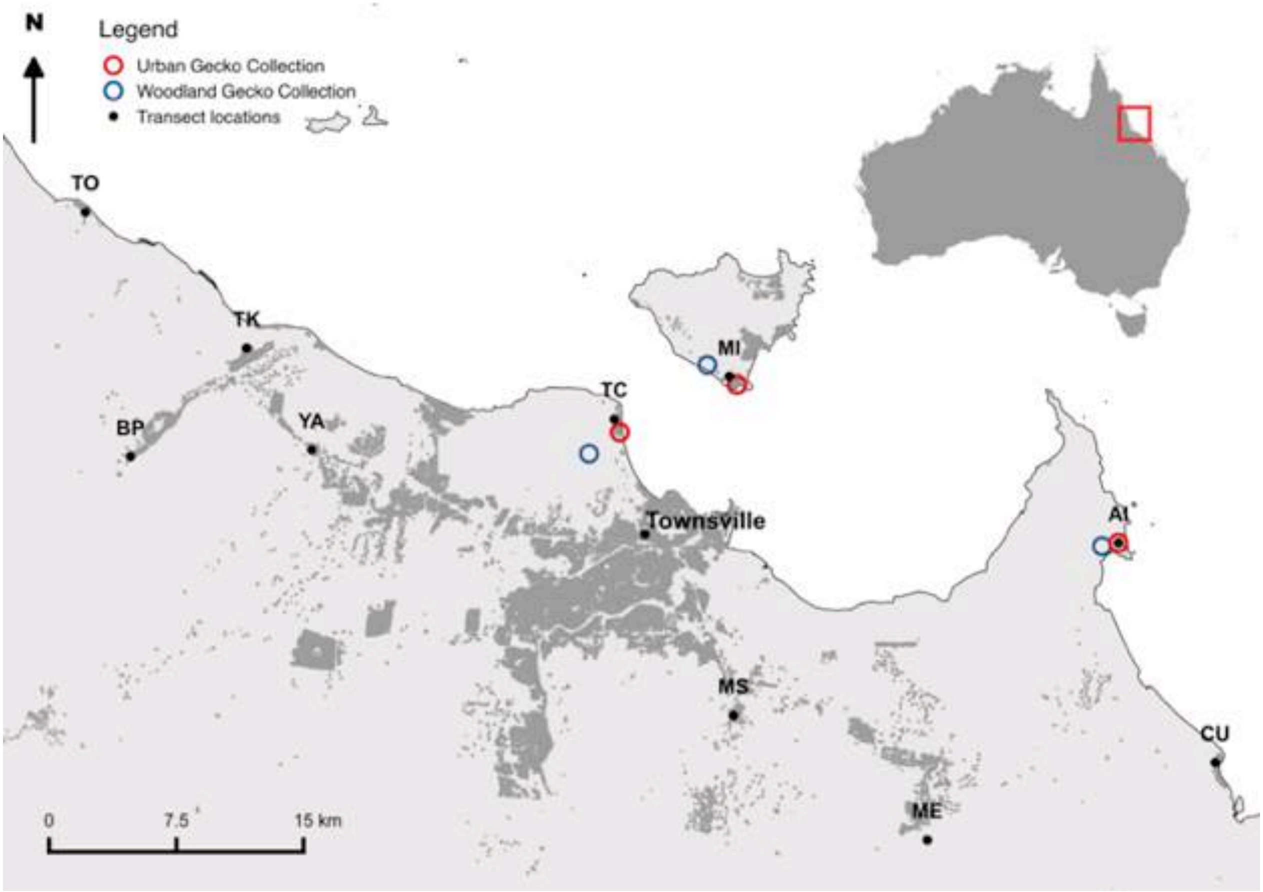


Figure 1. Map of invasion fronts studied. Black dots indicate transect locations where field-measured gecko size was assessed over eight months. Open circles are the specimen collection locations in urban (red) and woodland (blue) habitats for the lab-measured morphology analyses. On the inset map of Australia the study area is highlighted in red.

Body Size Analyses

All analyses were conducted in R version 3.3.0 (R Core Team 2016) unless otherwise specified. There was significant sexual dimorphism in body size and shape; therefore, we ran all models separately for males and females. We used stepwise model selection, in which we first included interactions between all variables then excluded the interactions that were not significant based on P-values. We also validated fitted models by assessing the residual plots.

Separately for each sex, we used an Analysis of Variance (ANOVA) to assess whether SVL differed between habitats (urban or woodland) and location (the long-term transects). Sites were nested within each location.

Lab-measured Body Condition and Body Shape

Gecko Collection and Measurements

To collect detailed morphological data, *Hemidactylus frenatus* were collected from paired urban and woodland sites, located at or near three of the long-term transects (Fig. 1). These collection locations included the Town Common (urban N = 20, woodland N = 20), Magnetic Island (urban N = 19, woodland N = 22), and the Australian Institute of Marine Science (AIMS, urban N = 20, woodland N = 18). All woodland geckos were located at least 500 m from the urban edge and we used the same techniques for gecko capture and sex determination as outlined above. These geckos were also weighed using a 10 g Pesola spring balance. Gecko collection was completed between 24 February and 9 March 2015.

Hemidactylus frenatus from the paired collection sites were humanely killed using tricaine methanesulfenate (MS222) (as per, Conroy et al., 2009 and James Cook University approval number A1801). Specimens were set in a container with 100% ethanol (ETOH) for 4 hours, before being transferred to a 75% ethanol solution, where they remained until measurements were taken. We used digital calipers to measure morphological characteristics, including snout-vent length (SVL), head length, head width, inter-limb length, forelimb length and hindlimb length of geckos (Supp. Fig. 2). Tail condition was recorded as ‘regenerated’ or ‘original’, and tail width was measured at 4 mm from the base of the tail as an alternative body condition index to weight relative to SVL. Tail base width indicates condition based on fat storage in the tail (Dial and Fitzpatrick 1981, Cooper et al. 2004). The same person (LKB) measured all geckos to ensure consistency in measurements.

Body Condition Analyses

We assessed body condition of the geckos collected from paired urban and woodland sites using two different indices of body condition for each sex: 1. a body condition index generated by taking the residuals from a linear regression between mass and SVL; and 2. a body condition index derived from the residuals of a linear regression between tail width and SVL. We used these body condition indices as response variables in two separate ANOVAs. In each model the predictor variables were habitat (urban or woodland), location (Magnetic Island, Town Common or AIMS), and tail condition (original or regenerated).

Body Shape Analyses

Body measurements (i.e., head width, head length, forelimb length, hindlimb length and interlimb length) were first corrected for gecko size by taking the residuals of a linear regression between each body measurement (e.g., Head length) and SVL separately for each sex. These size-corrected measurements were then used as response variables in multivariate linear models (one model for each sex) with predictor variables of habitat and location. These analyses were done within the ‘car’ package in R using type III sums of squares (Fox and Weisberg 2011). To visualise how different factors affected body shape we obtained canonical variates (CVs) of the exact same model using SAS

Studio University Edition Software (SAS Institute 2014).

Lab-measured Toe Morphology

Toe Morphology Measurements

A stereo microscope (Leica M165C) was used to photograph the underside of the fourth front and rear toe on the left side of each gecko (all photos were taken at 0.63 magnification) (Fig. 2). The Leica™ Application Suite was then used to place scales on these images for analyses.

Lamellae on the underside of front and rear toes were counted. Scales that were visibly enlarged and paired were classified as lamellae (Supp. Fig. 3). Lamellae on each digit were counted twice, and if the two counts matched, this number was used for analyses. If the counts were different, the lamellae were counted a third time, and the final number of lamellae was derived from the two consistent counts.

The program tpsDig (Rohlf 2013) was used to place landmarks on photos of gecko toes. Here, we used two different landmark configurations (Fig. 2). The first landmark configuration was designed to capture variation in entire toe size and shape. The second configuration was used to capture variation in size and shape of the four terminal lamellae, or main toepad. Together these provide relative lamellae size, as well as shape of the main toepad, which is likely to be very important for gripping.

Figure 2. The two landmark configurations on the underside of gecko toes designed to capture variation in the size and shape of the entire toe (red) and the main toepad (yellow). The yellow and red dots have been offset to make them both visible but the landmarks themselves were coincident.

Toe Morphology Analyses

Twelve traits were examined in eight separate analyses to assess whether toe morphology varies with habitat and collection location: the number of lamellae on front and rear toes (1), the length of front and rear toepads (2), the area of the front and rear whole toes (3) and front and rear toepads (4), the shape of the front (5) and rear (6) whole toes, and the shape of the front (7) and rear (8) toepads. Only the fourth front toe and fourth rear toe were measured. The whole toe was measured as the entire lamellae-bearing section of the toe, while the toepad was measured as the terminal four lamellae (Fig. 2).

We used multivariate linear models to assess whether habitat or location affected the number of lamellae on *H. frenatus* separately for each sex. In these analyses the number of lamellae on both the front and rear toe were used as the response variables, and habitat, location and SVL were used as predictor variables. These analyses were conducted in the ‘car’ package using type III sums of squares.

Landmark analyses of toe size and shape were carried out in the Geomorph Package (version 3.0.1) within R (Adams and Otárola-Castillo 2013). We ran generalised Procrustes analyses in Geomorph to generate centroid size and Procrustes coordinates (i.e., Procrustes residuals) for each landmark configuration. Centroid size is calculated as square root of the sum of squared distances between each landmark and the centroid of the configuration, and is a measure of the total area within the landmarks (Langerhans et al. 2004). Procrustes residuals are estimated using a generalised least squares criterion to centre, scale and rotate the original landmark configurations (Collyer et al. 2015). After this procedure the only remaining variation between specimens is due to shape, as the effects of size, orientation and position have been removed. Lamellae are paired structures (Fig. 2); therefore, we corrected any asymmetry in our shape data prior to analyses using the ‘bilat.symmetry’ function.

Length of the main toepad was calculated as the distance between the terminal landmark and the landmark at the base of the toepad (Fig. 2). This calculation was done on the raw landmark configurations, and toepad length was analysed using a multivariate linear model within the ‘car’ package (Fox and Weisberg 2011) and type III sums of squares. Response variables were front toepad length and rear toepad length. Habitat, location and SVL were the predictor variables.

To investigate changes in toe area we used multivariate linear models to analyse front and rear toe size in the same analyses. Response variables for the two separate models were: 1. centroid size of the entire front and rear toes, and 2. centroid size of the front and rear toepads. Predictor variables for both models were habitat, location and SVL. We conducted these analyses within the ‘car’ R package (Fox and Weisberg 2011) and used type III sums of squares.

Separately for each sex, we assessed whether toe shape varied between habitats or locations using non-parametric MANOVAs (npMANOVAs) with randomised residual permutation procedures (10,000 permutations) within the Geomorph R package version 3.0.1 (Adams and Otárola-Castillo 2013, Collyer et al. 2015). The response variables for each model were the symmetric Procrustes coordinates. The natural log of centroid size was included as a covariate in analyses to assess shape changes due to size (i.e., static allometry, Booksmythe et al. 2016). These analyses were run separately for the two different landmark configurations, and for both the front and rear toes.

Results

Field-measured body size

Habitat significantly affected the size (SVL) of male geckos, with males from woodland environments being significantly smaller than those from urban areas (Habitat effect: $F_{1,256} = 5.48$, $P = 0.02$; Fig. 3). Location did not affect male body size (Location effect: $F_{9,256} = 1.46$, $P = 0.16$; Site nested within Location: $F_{29,256} = 1.27$, $P = 0.17$). Female size did not vary between habitats (Habitat effect: $F_{1,242} = 0.18$, $P = 0.67$; Fig. 3), but did vary between locations (Location effect: $F_{9,242} = 2.53$, $P < 0.01$; Site nested within Location: $F_{28,242} = 1.92$, $P < 0.01$).

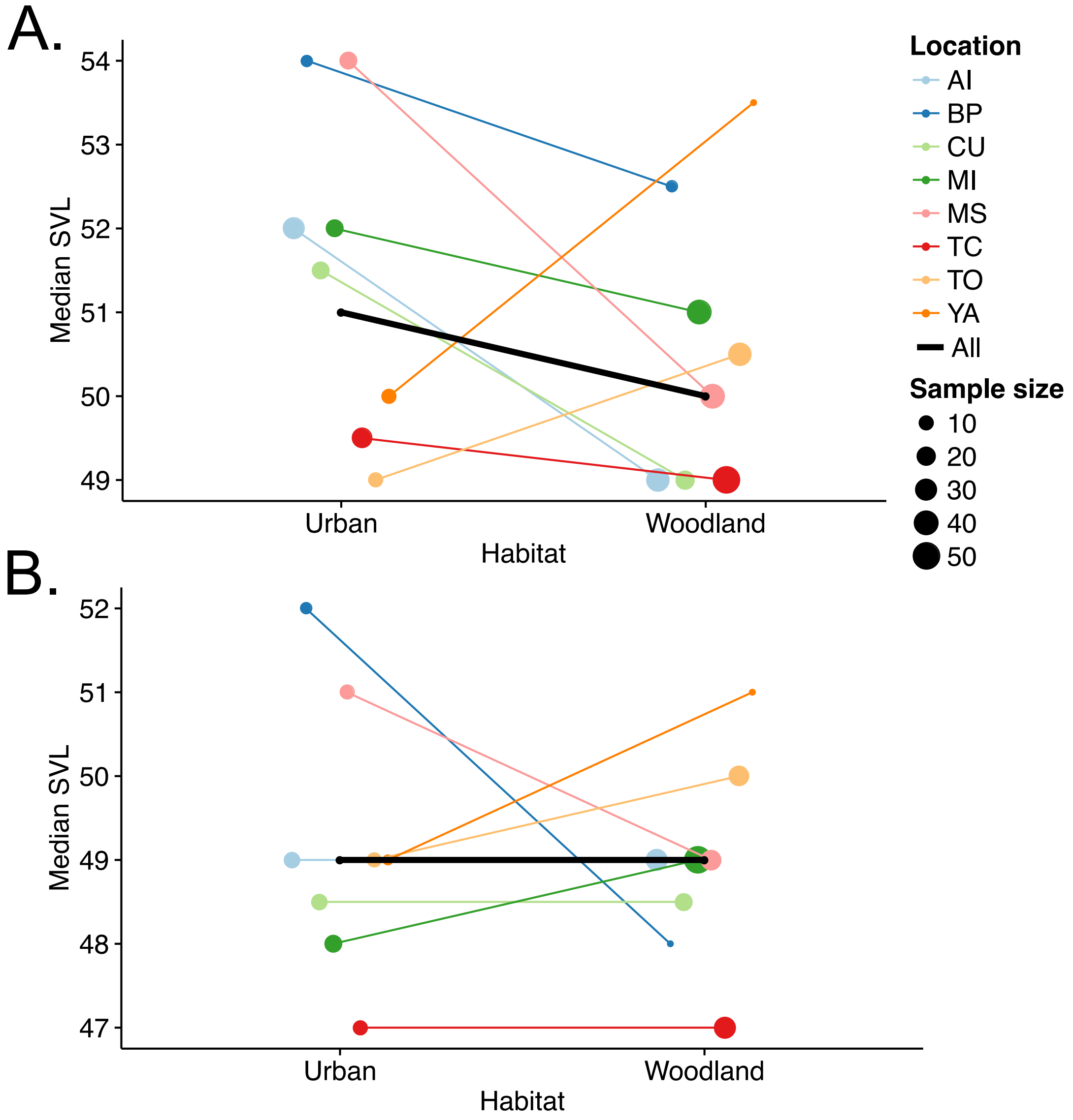


Figure 3. The median snout-vent length (SVL) of (A) male and (B) female geckos in urban and woodland habitats across eight locations (the long-term transects). The black line represents the median across all locations and the size of coloured circles indicates the total number of geckos measured in each habitat.

Lab-measured Body Condition and Body Shape

Body Condition

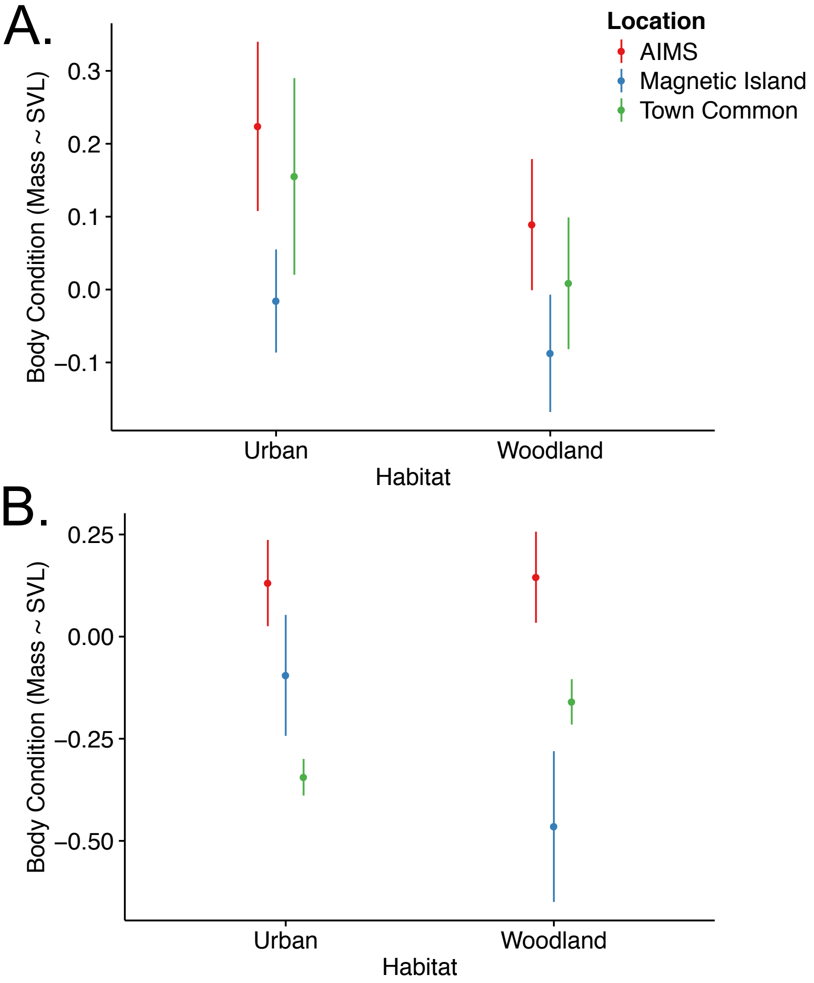


Figure 4. Mean body condition based on mass ± standard error of (A) male, and (B) female, *H. frenatus* per collection location.

We found no significant difference in body condition between urban and woodland populations, though there was a trend toward males in lower condition in woodland populations. This was true for body condition based on relative mass (Habitat effect: male: $F_{1,57} = 3.14$, $P = 0.08$; female: $F_{1,42} = 0.44$, $P = 0.51$), and for body condition based on tail width (Habitat effect: male: $F_{1,61} = 3.68$, $P = 0.06$; female: $F_{1,44} = 0.24$, $P = 0.63$). Body condition based on relative mass significantly differed between the three collection locations for both males (Location effect: $F_{2,57} = 3.39$, $P = 0.04$; Fig. 4A) and females (Location effect: $F_{2,42} = 5.45$, $P < 0.01$; Fig. 4B), as did male body condition based on tail width (Location effect: $F_{2,61} = 4.26$, $P = 0.02$). Female body condition based on tail width, however, was similar between the collection locations Location effect: $F_{2,44} = 0.91$, $P = 0.41$).

Tail condition (regenerated or original) affected male body condition based on mass (Tail condition effect: $F_{1,57} = 5.07$, $P = 0.03$) and males with a regenerated tail were in significantly poorer condition than those with an original tail (Post-hoc comparison: $t = 2.25$, $P = 0.03$). Tail condition did not affect body condition based on tail width for males (Tail condition effect: $F_{1,61} = 0.41$, $P = 0.53$), or females (Tail condition effect: $F_{1,44} = 0.02$, $P = 0.88$), and did not affect female body condition based on mass ($F_{1,42} = 2.60$, $P = 0.12$).

Body Shape

Habitat did not directly affect body shape of males (Habitat effect: $F_{5,58} = 0.81$, $P = 0.55$; Table 1). Location, however, significantly affected male body shape (Location effect: $F_{10,118} = 2.30$, $P = 0.02$), as did the interaction between location and habitat (Habitat*Location effect: $F_{10,118} = 2.44$, $P = 0.01$; Fig. 5). Body shape appeared to be similar between males from the three urban populations, but differed amongst the woodland populations (Fig. 5). These differences can be visualised using canonical variates. For males, the first axis of variation (CV1) accounted for 96% of differences in body shape and was positively weighted by forelimb length (0.32) and negatively weighted by hindlimb length (- 0.35) and head width (-0.20). The body shape of females, by contrast, was similar between habitats (Habitat effect: $F_{5,43} = 0.56$, P = 0.73; Table 1), and locations (Location effect: $F_{10,88} = 1.43$, P = 0.18; Table 1).

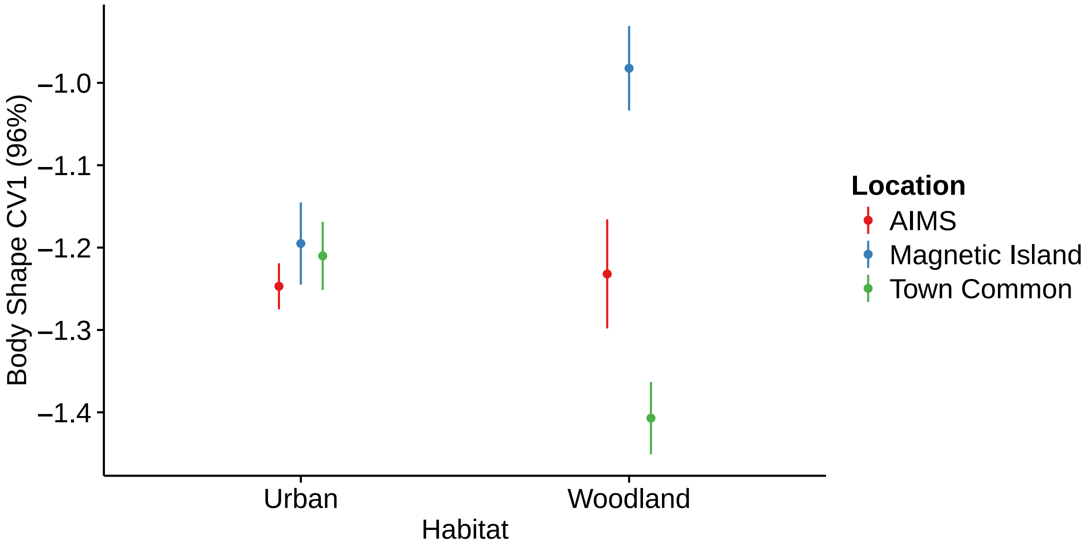


Figure 5. Differences in male body shape between the three woodland populations. CV1 is positively correlated with forelimb length (0.32), and negatively correlated with hindlimb length (-0.35) and head width (-0.20).

Table 1. Factors affecting body shape of male and female *H. frenatus*

Response	Sex	Parameter	Pillae	Wilks	f value	P-value
Residuals (Body measurements ~ SVL)	Male	Habitat	0.05	0.57	0.57	0.72
		Location**	0.35	2.61	2.50	<0.01
		Habitat.Location*	0.34	2.64	2.44	0.01
	Female	Habitat	0.07	0.93	0.62	0.68
		Location	0.28	0.73	1.43	0.18

* P ≤ 0.05
** P < 0.01
*** P < 0.0001

Toe Size and Number of Lamellae

The total number of lamellae on gecko toes (Supp. Fig. 3) was similar between geckos from urban and woodland environments (Habitat effect: male: $F_{2,55} = 1.46$, P = 0.24;

female: $F_{2,40} = 2.28$, $P = 0.12$). There was also no difference in number of lamellae between populations at different collection locations (Location effect: male: $F_{4,112} = 1.32$, $P = 0.27$; female: $F_{4,82} = 0.54$, $P = 0.71$), or geckos of different sizes (SVL effect: male: $F_{2,55} = 0.39$, $P = 0.68$; female: $F_{2,40} = 2.37$, $P = 0.11$).

Total toe size (centroid size of front and rear toes) was similar between populations from urban and woodland habitats (Habitat effect: male: $F_{2,58} = 1.09$, $P = 0.34$; female: $F_{2,42} = 2.13$, $P = 0.13$), and different locations (Location effect: male: $F_{4,118} = 0.69$, $P = 0.60$; female: $F_{4,86} = 0.37$, $P = 0.83$). As expected, there was a positive relationship between toe size and SVL for both males (SVL effect: $F_{2,58} = 64.99$, $P < 0.001$) and females (SVL effect: $F_{2,42} = 27.09$, $P < 0.001$).

Male toepad size (i.e., area covered by the four terminal lamellae; Fig. 2) was affected by habitat (Habitat effect: $F_{2,58} = 3.37$, $P = 0.04$; Fig. 6A), and post hoc tests revealed that males from woodland environments had significantly larger toepads than those collected from urban habitats ($t = 2.07$, $P = 0.04$; Fig. 6A). Male toepad size was similar between collection locations (Location effect: $F_{4,118} = 1.34$, $p = 0.26$; Fig. 6A), and there was a positive relationship between toepad size and SVL for male geckos (SVL effect: $F_{2,58} = 107.23$, $P < 0.001$).

Female toepad size was not affected by habitat (Habitat effect: $F_{2,42} = 0.97$, $P = 0.39$; Fig. 6B), but varied between locations (Location effect: $F_{4,86} = 3.49$, $p < 0.01$; Fig. 6B), and toepad size increased with gecko size (SVL effect: $F_{2,42}= 40.89$, $P < 0.001$).

Toepad length was similar between urban and natural habitats for both males (Habitat effect: $F_{2,58} = 2.73$, $P = 0.07$) and females (Habitat effect: $F_{2,42} = 0.95$, $P = 0.39$). Toepad length varied between collection locations for females (Location effect: $F_{4,86} = 2.52$, $P < 0.05$), but not males (Location effect: $F_{4,118} = 0.25$, $P = 0.91$), and SVL positively affected toepad length of both males (SVL effect: $F_{2,58} = 48.61$, $P < 0.001$) and females (SVL effect: $F_{2,42} = 26.60$, $P < 0.001$).

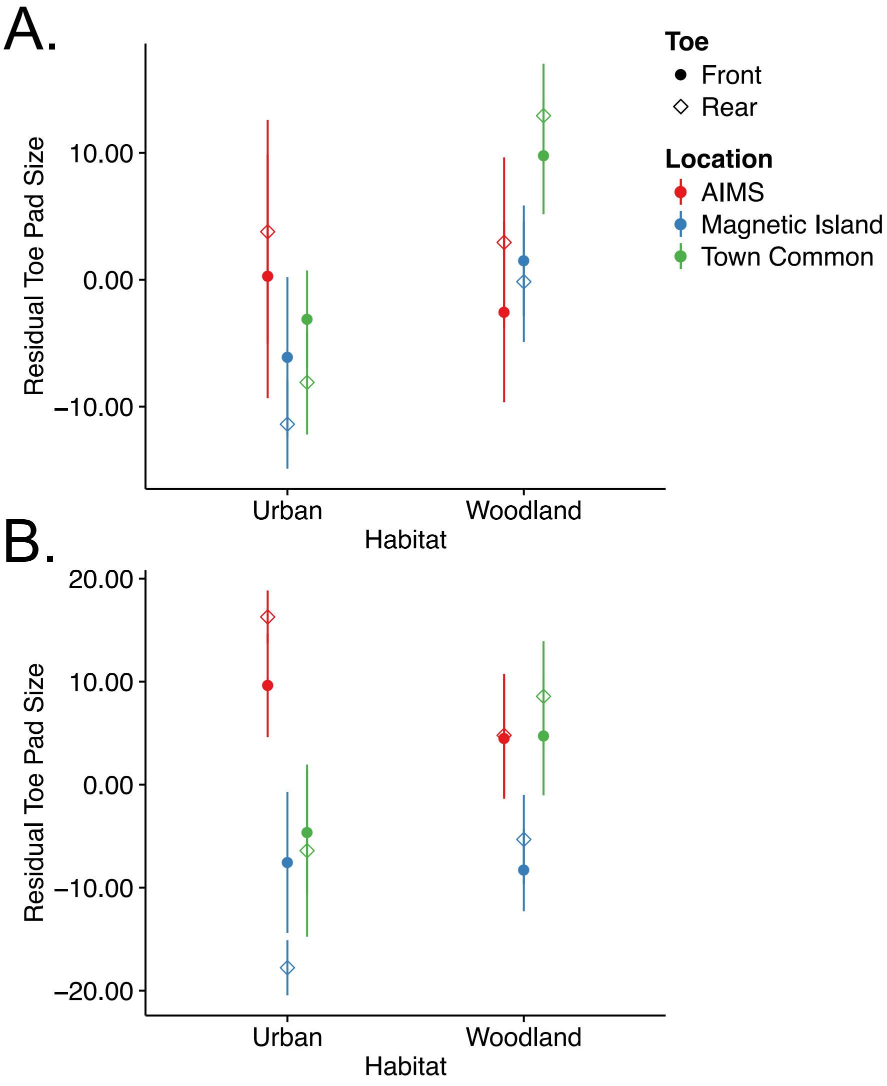


Figure 6. Relative size of the front (closed circles) and rear (open circles) toepads of (A) male and (B) female *H. frenatus*. Toepad size has been corrected for body size by taking the residuals of a linear regression with SVL.

Toe Shape

In general, gecko toe shape exhibited static allometry, with shape changing significantly depending on centroid size (Table 2.; Supp. Fig. 4). The only exception to this is the shape of front and rear toepads of female geckos (Centroid size effect: front: $F_{1,44}$ = 0.44, P = 0.82; rear: $F_{1,45}$ = 1.10, P = 0.33). This means that although size affects the overall shape of female toes (Centroid size effect: front: $F_{1,44}$ = 12.79 P < 0.001; rear: $F_{1,45}$ = 23.91, P < 0.001), the shape of the terminal toepad remains constant with size.

Table 2. Factors affecting overall toe shape of A. male and B. female *H. frenatus*

Model		Parameter	df	SS	MS	F	P-value
A. Male	Front Toe Shape	Centroid size (log)**	1	0.04	0.35	7.17	< 0.01
		Location	2	0.02	0.01	1.90	0.11
		Habitat*	1	0.02	0.02	3.15	0.03
		Habitat.Location**	2	0.04	0.02	3.78	< 0.01
	Rear Toe Shape	Centroid size (log)**	1	0.40	0.04	8.20	< 0.01
		Location	2	0.02	0.01	2.16	0.06
		Habitat	1	0.00	0.00	0.39	0.74
B. Female	Front Toe Shape	Centroid size (log)***	1	0.06	0.06	12.79	< 0.001
		Location*	2	0.03	0.01	3.29	0.01
		Habitat	1	0.01	0.01	1.17	0.26
	Rear Toe Shape	Centroid size (log)***	1	0.12	0.12	23.91	< 0.001
		Location	2	0.02	0.01	1.87	0.10
		Habitat	1	0.00	0.00	0.94	0.35

* P ≤ 0.05
** P < 0.01
*** P < 0.001

Male front toe shape varied between urban and woodland habitats (Habitat effect: $F_{1,57}$ = 3.15, P = 0.03), and although location alone did not affect overall toe shape (Location effect: $F_{2,57}$ = 1.90, P = 0.11), there was a significant interaction between habitat and location (Location*Habitat effect: $F_{2,57}$ = 3.78, P < 0.01, Fig. 7). Pairwise comparisons highlighted significant differences in toe shape (angles between slope vectors) between geckos from urban and woodland habitats on Magnetic Island (Z = 2.86, P < 0.01;

Supp. Table 2), but none of the other collection locations. Nonetheless, there was broad variation in toe shape between locations, with toe shape of urban males from Magnetic Island being significantly different to that of urban geckos from the Australian Institute of Marine Science ($Z = 2.63$, $P < 0.01$; Supp. Table 2). The rear toe shape males was unaffected by habitat (Habitat effect: $F_{1,60} = 0.39$, $P = 0.74$), or location (Location effect: $F_{2,60} = 2.16$ $P = 0.06$).

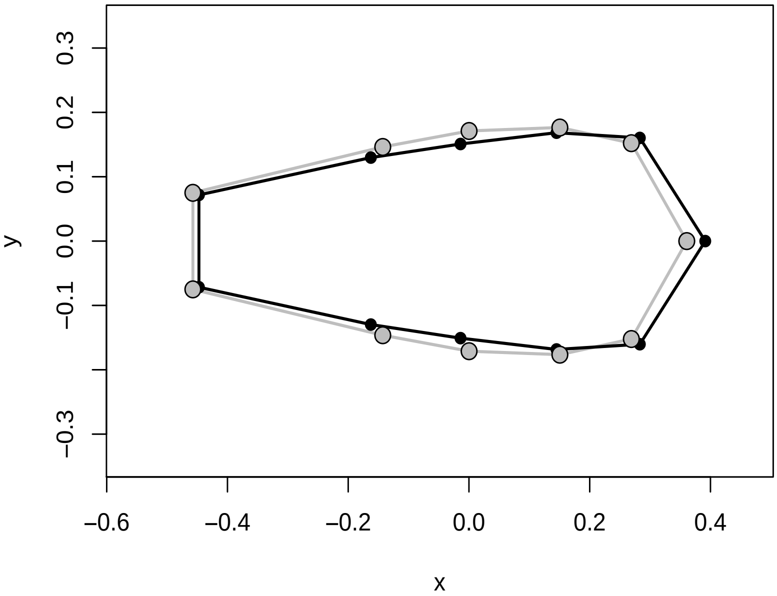


Figure 7. Mean front toe shape of urban (grey) and woodland (black) male *H. frenatus* at Magnetic Island. Differences have been magnified three times in order to aid visualisation.

Female front toe shape was similar in woodland and urban environments (Habitat effect: $F_{1,44} = 1.17$, $P = 0.26$;), but varied between collection locations ($F_{2,44} = 3.29$, $P < 0.01$). The rear toe shape of females was unaffected by habitat (Habitat effect: $F_{1,45} = 0.94$, $P = 0.35$), or location (Location effect: $F_{2,45} = 1.87$, $P = 0.10$),

The shape of male toepads was unaffected by habitat (Habitat effect: front: $F_{1,59} = 1.59$, $P = 0.14$; rear: $F_{1,60} = 0.76$, $P = 0.51$; Supp. Table 1), but location affected both the front and rear toepad shape of male geckos (Location effect: front: $F_{2,59} = 3.73$, $P < 0.01$; rear: $F_{2,60} = 2.61$, $P = 0.01$, Supp. Fig. 5). Female toepad shape was not affected by habitat (Habitat effect: front: $F_{1,44} = 0.43$, $P = 0.79$; rear: $F_{1,45} = 0.86$, $P = 0.43$; Supp. Table 1), and location affected the shape of female front toepads (Location effect: $F_{2,44} = 2.70$, $P = 0.01$; Supp. Fig. 5), but had no effect on rear toepad shape (Location effect: $F_{2,45} = 0.70$, $P = 0.66$).

Discussion

Morphological divergence can facilitate establishment and spread of species in novel habitats (Phillips et al. 2006, Zuk et al. 2006). Such changes may be particularly likely to arise in populations transitioning the urban–natural interface, because of the stark differences between urban and natural habitats. We investigated whether morphological divergence occurs upon colonisation of natural environments by a human-associated species, the Asian house gecko (*Hemidactylus frenatus*) across semi-independent invasion fronts in northern Australia. We assessed whether 1) the different woodland populations show morphological trait shifts from urban populations in the same direction, or 2) woodland populations show more variation in traits across invasion fronts than urban populations because woodland environments are more variable than urban environments.

This study provides evidence of differences in morphology between urban and woodland populations of *H. frenatus*. We found that some traits differed consistently between urban and natural environments, but for multiple morphological traits, the direction of change in woodland environments differed between the invasion fronts studied. This indicates trait divergence associated with colonisation of different natural areas. We also found the sexes responded differently to the shift in habitat.

We observed sexual dimorphism in body size of *H. frenatus*, with males being larger than females (Fig. 3), in accordance with other studies (Cameron et al. 2013). Female body size was similar between urban and natural environments, but males from woodland habitats were on average smaller than those from urban areas (Fig. 3). There are a number of potential explanations for this observation. First, it is possible that males have shorter lifespans in natural environments. We believe this is unlikely, because body condition did not differ between habitats, indicating that *H. frenatus* are doing well in natural environments, and female body size was similar in urban and woodland habitats. The second possibility is that larger bodies are not as beneficial for males in natural environments because of lower population density in these habitats. Aggressive interactions are common between male *H. frenatus* (Dame and Petren 2006, Cameron et al. 2013); however, in woodland habitats, lower overall gecko density means that encounters between males may be less frequent (Barnett et al. 2017), and attaining larger body sizes may not confer a significant advantage for males in natural environments. Finally, it is possible that a detection bias has caused this pattern. Large male geckos may dominate the prime foraging habitat in urban environments making them more detectable than smaller males, which may be excluded to suboptimal habitat where they are harder to find. In natural environments resources are more evenly distributed (Petren and Case 1998), and males of all size classes may be similarly detectable.

Sexual dimorphism and sex-specific morphological responses are common in lizards (Herrel et al. 1999, Johnson et al. 2005, Dill et al. 2013), and may arise through sex-specific differences in behaviour and ecology (Dill et al. 2013). In *H. frenatus*, for example, territorial males likely experience greater selection for traits associated with aggressive competition (e.g., bite force), while for females, selection may favour other traits, such as sprint speed (Cameron et al. 2013). It is, therefore, possible that similar factors drive the sex-specific morphological responses observed in the current study.

Like male body size, male toepad size also differed consistently between urban and woodland habitats and males in woodland areas had significantly larger toepads than those from urban environments (Fig. 6A). This result was unexpected because larger toepads are likely to have more individual setae and should be more effective at gripping smooth surfaces (Autumn et al. 2000). Therefore, one would expect larger toepads on geckos in urban areas where there are more smooth surfaces than in natural habitats (Winchell et al. 2016; LKB, pers. obs.). Future work could investigate this pattern through detailed assessment of the characteristics of urban and woodland habitats (e.g., the availability of different foraging surfaces) at the different invasion fronts, and through laboratory studies to investigate the influence of toepad size on *H. frenatus* performance (e.g. gripping ability or sprint speed) on different substrates.

While male body size and male toepad size changed consistently between urban and woodland environments across different invasion fronts, these were the only traits that changed consistently between habitats. Some traits were not affected by either habitat or invasion front (e.g. female body shape and the number of lamellae on gecko toes) and for other traits the direction of divergence in natural habitats differed depending on the location being examined. Some of these traits showed more variation in the woodland populations than in the urban populations, an expectation based on the likely greater variability in natural than urban environments. For example, male body shape was similar in urban areas, but varied between the three woodland populations (Fig. 5). Male toe shape was also highly dependent on location, and divergence in toe shape was observed between urban and woodland populations at Magnetic Island (Table 2; Fig. 7), but at neither of the other collection locations.

Similarities in morphology between the three urban areas were expected, because anthropogenic habitats are less variable than natural environments (Lei and Booth 2014, Winchell et al. 2016). Therefore, selection pressures may be similar in the different urban locations, but differ between the three woodland locations. It is, however, important to note that some traits varied between locations irrespective of habitat. Female front toepad shape, for example, differed between geckos from the Town Common and Australian Institute of Marine Science invasion fronts (Supp. Fig. 5), while being unaffected by habitat. Furthermore, body condition varied between the three different locations (Fig. 4), suggesting that although these areas were all within 30 km of each other, there may be broad differences between them. Variation in morphology between the three invasion fronts may have arisen through founder events, be a response to location-specific environmental differences (e.g., differences in precipitation between the invasion fronts), or be due to different establishment histories of *H. frenatus* at the three invasion fronts.

On the three invasion fronts that we used to study foot morphology and body shape, *H. frenatus* are estimated to have established in urban areas between 1980 and 1989

(Barnett et al. 2017). Previous work indicates that the time taken for this species to spread into woodland habitats is dependent on propagule pressure (i.e., size of the urban population) and establishment time (Barnett et al. 2017), and these factors might also contribute to morphological differences between invasion fronts. Furthermore, if morphological trait shifts are adaptive, the capacity for adaptation is likely affected by a complex interplay between the strength of selection and the level of gene flow from surrounding areas (Fitzpatrick et al. 2015), and these factors will vary between the three invasion fronts studied. It is, nonetheless, clear that fine scale morphological divergence can occur within the timescales studied (i.e., over 26 years), whether it be through adaptive evolution or phenotypic plasticity (Losos et al. 1997, Langford et al. 2014).

Future research should assess whether the changes in the morphological traits we observe here are the result of phenotypic plasticity or adaptive divergence (e.g., conduct common garden experiments and raise the offspring in different environments). It is likely that certain morphological traits have higher heritability than others (consider toe pad size or relative limb length versus body size) and it would be interesting to investigate this in more detail in the current system. Future work should also expand the sampling to include more sites and regions, quantify physical environmental characteristics at each site (e.g., foraging surfaces), quantify the microhabitat use in different habitats, test functional morphology in experiments (e.g., sprint speed and gripping ability on different surfaces), and include common garden experiments to test the mechanism behind key morphological shifts between urban and woodland populations.

Conclusion

Hemidactylus frenatus is a globally widespread invasive species that is highly adapted to life in urban areas. In this study we show that morphological trait shifts are associated with colonisation of natural environments by this species. However, trait shifts were complex. Some shifts were restricted to one sex, and many traits shifted in different ways at different invasion fronts. For a number of these traits, variation between sites was greater for woodland than urban populations, suggesting that invasion of natural environments may involve different morphological changes in different areas. Overall, our results provide evidence that morphological changes can occur as human-associated species colonise natural environments, but that these trait shifts are not consistent. Such divergent outcomes contrast to the expectation of convergence when organisms from natural habitats invade urban environments.

Acknowledgments

We thank Kirilly O’reilly, Roderick Budd, Dylan Tegtmeier and Nantida Sutummawong for help with field and laboratory work. Thanks also to Kelsey Hosking, Stewart Macdonald and Stephen Zozaya who gave valuable advice during data analysis. Thanks to Queensland Parks & Wildlife Service, the Australian Institute of Marine Science, and private residents for access to sites.

References

Adams, D. C. and Otárola-Castillo, E. 2013. Geomorph: An R package for the collection and analysis of geometric morphometric shape data. - Methods Ecol. Evol. 4: 393–399.

Alberti, M. et al. 2003. Integrating humans into ecology: Opportunities and challenges for studying urban ecosystems. - Bioscience 53: 1169–1179.

Autumn, K. et al. 2000. Adhesive force of a single gecko foot-hair. - Nature 405: 681–685.

Barnett, L. K. et al. 2017. Going feral: Time and propagule pressure determine range expansion of Asian house geckos into natural environments. - Austral Ecol. 42: 165–175.

Bolger, D. T. et al. 1997. Breeding bird abundance in an urbanizing landscape in coastal southern California. - Conserv. Biol. 11: 406–421.

Booksmythe, I. et al. 2016. Fitness consequences of artificial selection on relative male genital size. - Nat. Commun. 7: 11597.

Cameron, S. F. et al. 2013. Sex-specific trade-offs and compensatory mechanisms: bite force and sprint speed pose conflicting demands on the design of geckos (*Hemidactylus frenatus*). - J. Exp. Biol. 216: 3781–3789.

Case, T. J. and Bolger, D. T. 1991. The role of introduced species in shaping the distribution and abundance of island reptiles. - Evol. Ecol. 5: 272–290.

Cole, N. C. et al. 2005. The need for enemy-free space: the impact of an invasive gecko on island endemics. - Biol. Conserv. 125: 467–474.

Collyer, M. L. et al. 2015. A method for analysis of phenotypic change for phenotypes described by high-dimensional data. - Hered. 115: 357–365.

Conroy, C. J. et al. 2009. Use of tricaine methanesulfonate (MS222) for euthanasia of reptiles. - J. Am. Assoc. Lab. Anim. Sci. 48: 28–32.

Cooper, W. E. et al. 2004. Ease and effectiveness of costly autotomy vary with predation intensity among lizard populations. - J. Zool. 262: 243–255.

Dame, E. A. and Petren, K. 2006. Behavioural mechanisms of invasion and displacement in Pacific island geckos (*Hemidactylus*). - Anim. Behav. 71: 1165–1173.

Davidson, A. M. et al. 2011. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. - Ecol. Lett. 14: 419–431.

Dial, B. E. and Fitzpatrick, L. C. 1981. The energetic costs of tail autotomy to reproduction in the lizard *Coleonyx brevis* (Sauria: Gekkonidae). - Oecologia 51: 310–317.

Dill, A. K. et al. 2013. Sexual dimorphisms in habitat-specific morphology and behavior in the green anole lizard. - J. Zool. 290: 135–142.

Fitzpatrick, S. W. et al. 2015. Locally adapted traits maintained in the face of high gene flow (G Grether, Ed.). - Ecol. Lett. 18: 37–47.

Fox, J. and Weisberg, S. 2011. An R companion to applied regression. - Sage.

Ghalambor, C. K. et al. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. - Funct. Ecol. 21: 394–407.

Hanley, K. et al. 1996. An experimental demonstration of exploitation competition in an ongoing invasion. - Ecology 77: 118–132.

Herrel, A. et al. 1999. Sexual dimorphism of head size in *Gallotia galloti*: Testing the niche divergence hypothesis by functional analyses. - Funct. Ecol. 13: 289–297.

Higham, T. E. and Russell, A. P. 2010. Divergence in locomotor performance, ecology, and morphology between two sympatric sister species of desert-dwelling gecko. - Biol. J. Linn. Soc. 101: 860–869.

Holway, D. A. and Suarez, A. A. V 1999. Animal behavior: an essential component of invasion biology. - Trends Ecol. Evol. 14: 328–330.

Hoskin, C. J. 2011. The invasion and potential impact of the Asian House Gecko (*Hemidactylus frenatus*) in Australia. - Austral Ecol. 36: 240–251.

Hulme, P. E. 2009. Trade, transport and trouble: managing invasive species pathways in an era of globalization. - J. Appl. Ecol. 46: 10–18.

Irschick, D. J. and Garland, T. 2001. Integrating function and ecology in studies of adaptation: investigations of locomotor capacity as a model system. - Annu. Rev. Ecol. Syst. 32: 367–396.

Johnson, J. et al. 2005. Allometry, sexual size dimorphism, and niche partitioning in the mediterranean gecko (*Hemidactylus turcicus*). - Southwertern Nat. 50: 435–439.

Jokimäki, J. and Huhta, E. 2000. Artificial nest predation and abundance of birds along an urban gradient. - Condor 102: 838–847.

Kolbe, J. J. et al. 2012. Climatic niche shift predicts thermal trait response in one but not both introductions of the Puerto Rican lizard *Anolis cristatellus* to Miami, Florida, USA. - Ecol. Evol. 2: 1503–16.

Kotler, B. P. et al. 1988. The effects of morphology and body size on rates of owl predation on desert rodents. - Oikos 53: 145–152.

Langerhans, R. B. et al. 2004. Predator-driven phenotypic diversification in *Gambusia affinis*. - Evolution (N. Y). 58: 2305–2318.

Langford, G. J. et al. 2014. Phenotypic Plasticity in the Relative Hind-Limb Growth of Lab-Reared *Anolis sagrei*: Replication of Experimental Results and a Test of Perch Diameter Preference. - J. Herpetol. 48: 228–232.

Lei, J. and Booth, D. T. 2014. Temperature, field activity and post-feeding metabolic response in the Asian house gecko, *Hemidactylus frenatus*. - J. Therm. Biol. 45: 175–80.

Lever, C. 2003. Naturalized reptiles and amphibians of the world. - Oxford University Press.

Losos, J. B. et al. 1997. Adaptive differentiation following experimental island colonization in Anolis lizards. - Nature 387: 70–73.

Møller, A. P. 2008. Flight distance of urban birds, predation, and selection for urban life. - Behav. Ecol. Sociobiol. 63: 63–75.

Mörtberg, U. and Wallentinus, H.-G. G. 2000. Red-listed forest bird species in an urban environment — assessment of green space corridors. - Landsc. Urban Plan. 50: 215–226.

Mukherjee, A. et al. 2011. Brazilian peppertree (*Schinus terebinthifolius*) in Florida and South America: evidence of a possible niche shift driven by hybridization. - Biol. Invasions 14: 1415–1430.

Petren, K. and Case, T. J. 1998. Habitat structure determines competition intensity and invasion success in gecko lizards. - Proc. Natl. Acad. Sci. U. S. A. 95: 11739–11744.

Phillips, B. L. et al. 2006. Invasion and the evolution of speed in toads. - Nature 439: 803.

Prentis, P. J. et al. 2008. Adaptive evolution in invasive species. - Trends Plant Sci. 13: 288–294.

R Core Team 2016. R: A language and environment for statistical computing.: R Foundation for Statistical Computing, Vienna, Au.

Rejmánek, M. and Richardson, D. M. 1996. What attributes make some plant species more invasive? - Ecology 77: 1655–1661.

Richardson, D. M. and Ricciardi, A. 2013. Misleading criticisms of invasion science: a field guide. - Divers. Distrib. 19: 1461–1467.

Rödder, D. et al. 2008. Predicting the potential distribution of two alien invasive Housegeckos (Gekkonidae: *Hemidactylus frenatus, Hemidactylus mabouia*). - North. West. J. Zool. 4: 236–246.

Rohlf, F. 2013. tpsDig, Thin-Plate Spline Digitising Software. in press.

Sætre, G. P. et al. 2012. Single origin of human commensalism in the house sparrow. - J. Evol. Biol. 25: 788–796.

SAS Institute 2014. SAS Studio University Edition. in press.

Shochat, E. et al. 2010. Birds in Urban Ecosystems: Population Dynamics, Community Structure, Biodiversity, and Conservation. - Urban Ecosyst. Ecol. Agron. Monogr. 55: 75–86.

Urban, M. C. et al. 2007. The cane toad's (*Chaunus* [*Bufo*] *marinus*) increasing ability to invade Australia is revealed by a dynamically updated range model. - Proc. R. Soc. B Biol. Sci. 274: 1413–1419.

Vervust, B. et al. 2007. Differences in morphology, performance and behaviour between recently diverged populations of *Podarcis sicula* mirror differences in predation pressure. - Oikos 116: 1343–1352.

Wikelski, M. 2005. Evolution of body size in Galapagos marine iguanas. - Proc. R. Soc. B Biol. Sci. 272: 1985–1993.

Winchell, K. M. et al. 2016. Phenotypic shifts in urban areas in the tropical lizard *Anolis cristatellus*. - Evolution (N. Y). 70: 1009–1022.

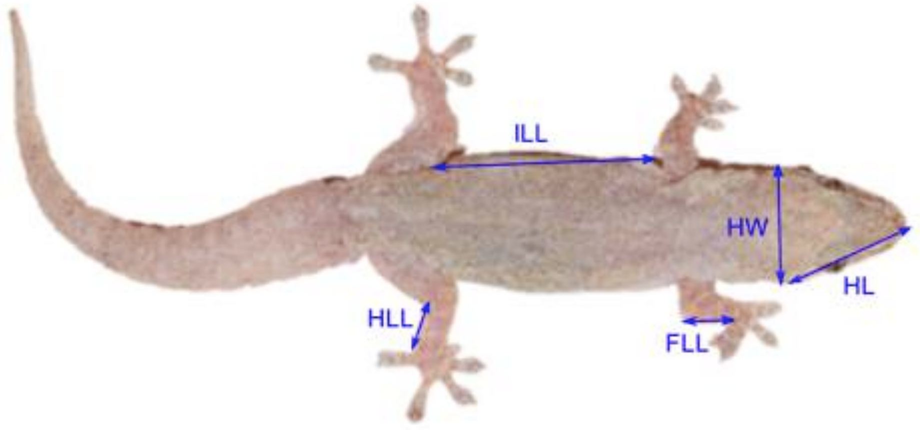
Zozaya, S. M. et al. 2015. Invasive house geckos are more willing to use artificial lights than are native geckos. - Austral Ecol. 40: 982–987.

Zuk, M. et al. 2006. Silent night: adaptive disappearance of a sexual signal in a parasitized population of field crickets. - Biol. Lett. 2: 521–4.

Chapter 3

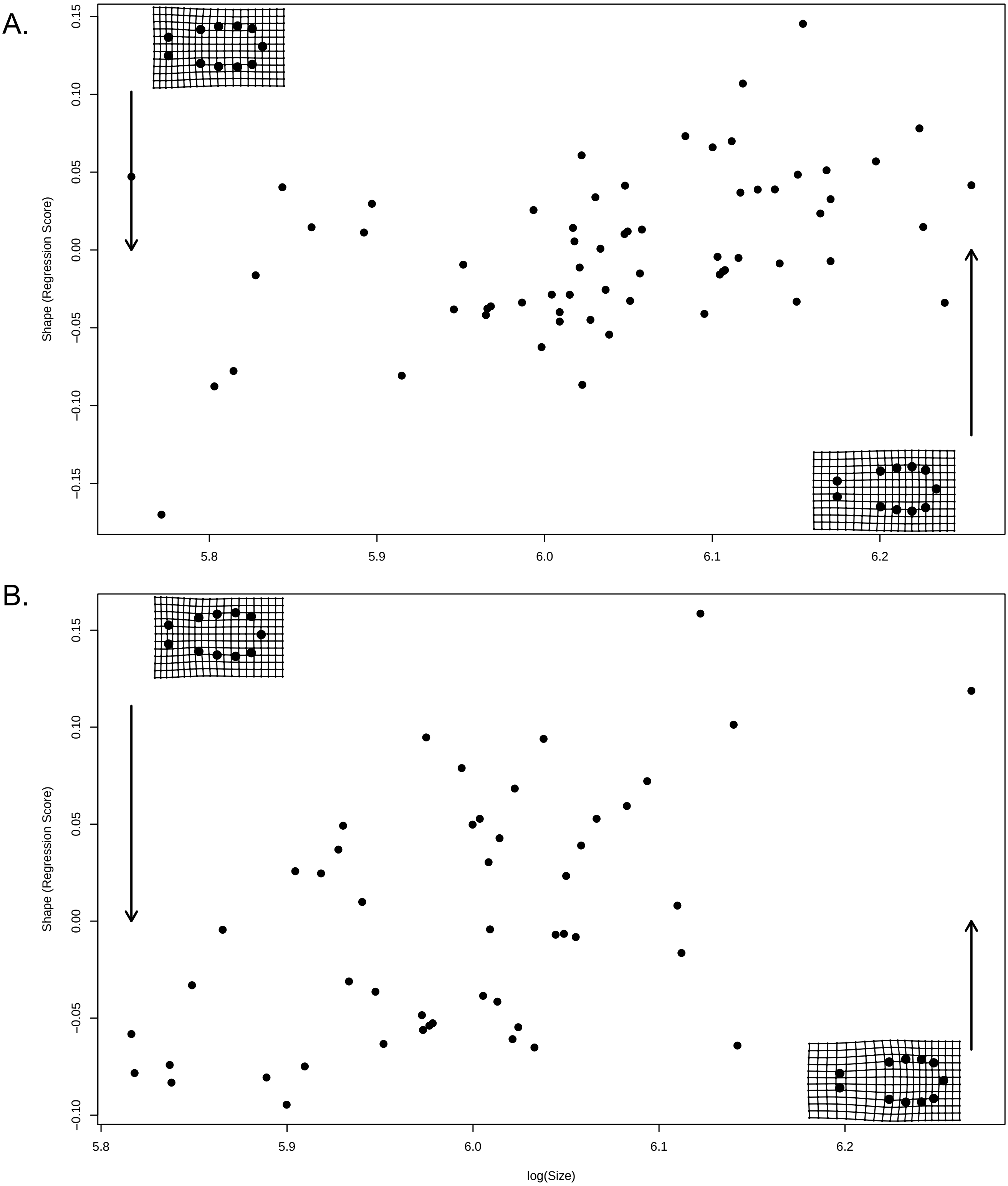
Supplementary Material

Supplementary Figure 1. Example of (A) urban and (B) woodland habitat studied.

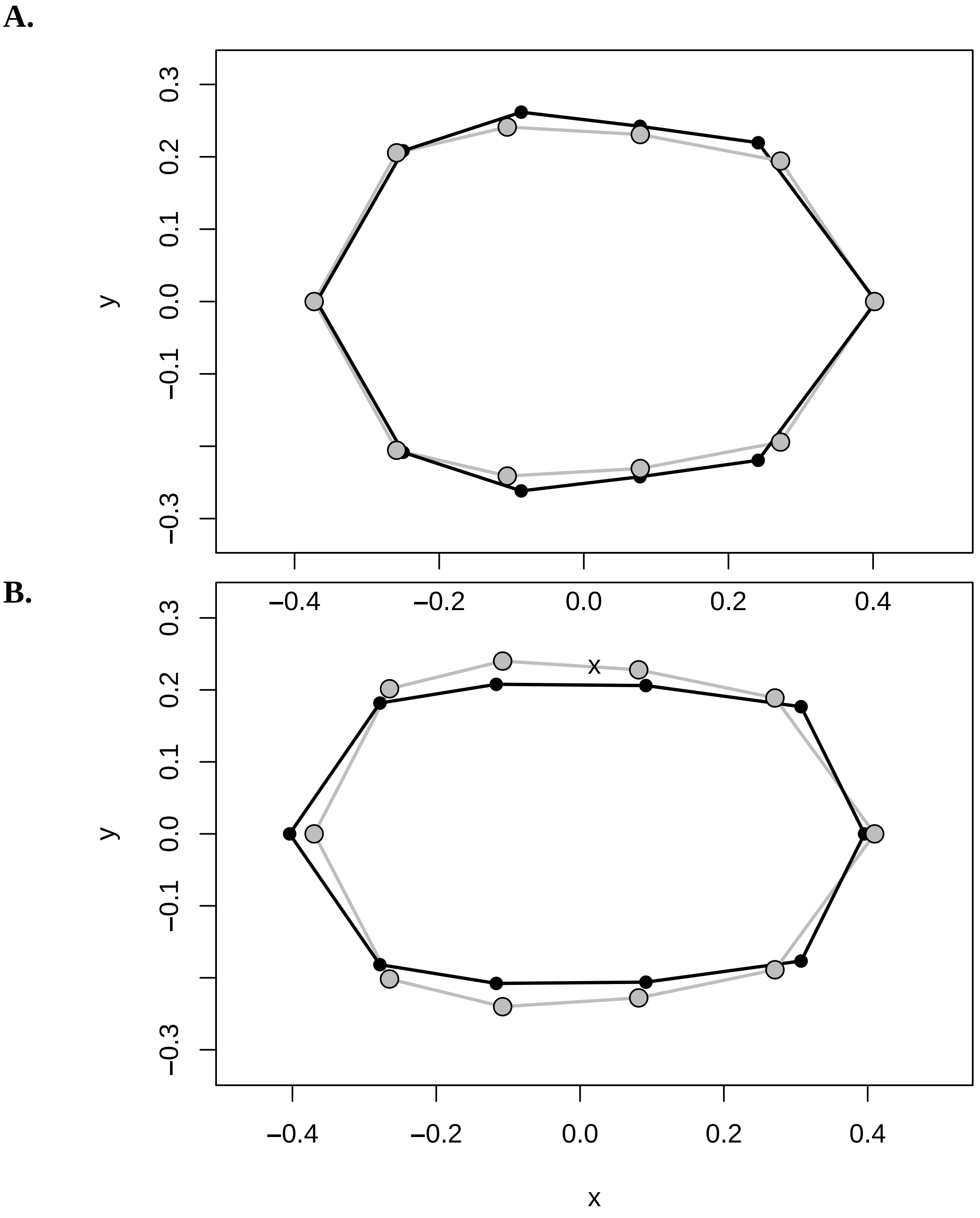


Supplementary Figure 2. Body shape measurements of geckos (ILL = Inter-limb length, HLL = Hind limb length, FLL = Fore limb length, HW = Head width, HL = Head length). Snout-vent length (SVL) was also measured but is not pictured here. Photo credit: Stephen Zozaya

Supplementary Figure 3. A *H. frenatus* toe with the sub-digital lamellae highlighted. All enlarged and paired scales were classified as lamellae for counts.



Supplementary Figure 4. Changes in *H. frenatus* front toe shape with centroid size (i.e. static allometry) for (**A)** Males and (**B)** Females



Supplementary Figure 5. The significant difference in mean shape of the front toepad between *H. frenatus* collected from The Australian Institute of Marine Science (grey) and the Town Common (black), for **A.** Males and **B.** Females. Shape differences have been magnified by three to aid visualisation.

Supplementary Table 1. Factors affecting toepad shape of geckos

Model		Parameter	df	SS	MS	F	P-value
A. Male	Front Toe Pad Shape	Centroid size (log)*	1	0.18	0.18	4.12	0.01
		Location**	2	0.03	0.02	3.73	< 0.01
		Habitat	1	0.01	0.01	1.59	0.14
	Rear Toe Pad Shape	Centroid size (log)**	1	0.02	0.02	5.72	< 0.01
		Location*	2	0.02	0.01	2.61	0.01
		Habitat	1	< 0.01	< 0.01	0.76	0.51
B. Female	Front Toe Pad Shape	Centroid size (log)**	1	< 0.01	< 0.01	0.44	0.82
		Location*	2	0.02	0.01	2.70	0.01
		Habitat	1	< 0.01	< 0.01	0.43	0.79
	Rear Toe Pad Shape	Centroid size (log)*	1	< 0.01	< 0.01	1.10	0.33
		Location	2	0.01	< 0.01	0.70	0.66
		Habitat	1	< 0.01	< 0.01	0.86	0.43

* P ≤ 0.05
** P < 0.01
*** P < 0.001

Supplementary Table 2. Differences in least squares means for front toe shape of male *H. frenatus*. Effect sizes (Z-values) are below the diagonal and associated P-values are above the diagonal. Significant P-values are in bold.

			P-values					
			Woodland			Urban		
			AI	MI	TC	AI	MI	TC
Effect Sizes (Z)	Woodland	AI		0.33	0.63	0.43	0.09	0.31
		MI	0.91		0.71	0.53	**0.005**	0.35
		TC	0.51	0.39		0.94	**0.011**	0.31
	Urban	AI	0.78	0.58	0.15		**0.001**	0.13
		MI	1.77	2.86	2.16	2.63		0.18
		TC	1.02	0.84	1.06	1.56	1.35	

Chapter 4.

Host specificity and the potential impact of parasites during range expansion of an invasive gecko

Louise K Barnett[1], Ben L Phillips[2], Allen CG Heath[3] Andrew Coates[2],
Conrad J Hoskin[1]

[1] Centre for Tropical Biodiversity & Climate Change, College of Science and Engineering, James Cook University, Townsville, Queensland 4811, Australia.
[2] School of Biosciences, University of Melbourne, Parkville, Victoria 3010, Australia.
[3] AgResearch Ltd, Hopkirk Research Institute, Palmerston North, 4442, New Zealand

17 March 2017

Manuscript for consideration in: *OIKOS*

Abstract

Host-parasite dynamics can play a fundamental role in both the establishment success of an invasive species and its impact on native wildlife. Parasites may switch between invasive and native hosts, and they may impact range expansion of the invasive. We addressed these issues on ten transects where Asian house geckos (*Hemidactylus frenatus*) are expanding from urban areas into natural environments. In the natural environments, *H. frenatus* co-occurs with, and at many sites now greatly out-numbers, native geckos. The parasites we assessed were native and introduced *Geckobia* mites and native *Waddycephalus* pentastomes. There was no evidence of mite transmission between native and invasive geckos, with native mite species found only on native geckos and invasive mite species restricted to the invasive host. This is despite the fact that *H. frenatus* and native geckos have occurred side-by-side at some sites for many years. In contrast, native *Waddycephalus* nymphs were commonly present in *H. frenatus,* demonstrating this parasite's capacity to utilise the invasive host. Prevalence of mites on *H. frenatus* decreased with distance from the urban edge, suggesting parasite release towards the invasion front; however, we found no evidence that mites affect *H. frenatus* body condition or lifespan. *Waddycephalus* was present at low prevalence in the bushland sites and, although its presence did not affect host body condition, our data suggest it may reduce host survival. The high relative density of *Hemidactylus* at our sites, and their capacity to harbour *Waddycephalus,* suggests there may be impacts on native geckos and snakes through parasite spillback.

Key words: parasites; *Hemidactylus frenatus*; range expansion; *Geckobia*, *Waddycephalus*

Introduction

Invasive species pose an increasing threat to global biodiversity (Floerl et al. 2009). While invasives have numerous direct effects—through predation, competition, or hybridization with native species (Menge 1972, O'Dowd et al. 2003, Wanless et al. 2007)—they may also have powerful indirect effects, by spreading parasites and diseases (Dunn and Hatcher 2015). Each introduced species brings an average of three parasite species with them from their native range (Torchin et al. 2003), and these parasites can have serious impacts on native species (Andreou et al. 2012). The impact of parasites during biological invasions is complex, involving interactions between native and introduced hosts and their respective parasite communities (Dunn et al. 2012).

Introduced host species may affect parasite prevalence in native hosts in two ways. First, introduced parasites may switch to novel native hosts, causing a 'spillover' of introduced parasites (Andreou et al. 2012). Naïve native species are often highly susceptible to introduced parasites, while invasive species, which have co-evolved with these parasites, may not be obviously affected (Andreou et al. 2012). Thus, spillover may have serious impacts on native hosts (Hudson and Greenman 1998). Second, over time, invasive hosts acquire native parasites in the new range (Torchin and Mitchell 2004). The presence of this new (often highly abundant) host, may cause a dramatic increase in native parasite abundance, which may affect native hosts through parasite 'spillback' (Kelly et al. 2009, Hartigan et al. 2011, Kelehear et al. 2013). Therefore, in order to understand the potential impact of introduced species on biodiversity it is important to assess the potential for parasite transmission between native and introduced hosts.

While invasive species do bring novel parasites and pathogens with them, they generally leave many of their natural parasites behind when they establish in their new range. This 'parasite release'—driven by founder events or a lack of intermediate hosts in the new range—may facilitate establishment and range expansion of invasive species (Torchin et al. 2003, Prenter et al. 2004, Dunn and Hatcher 2015). As well as this loss of parasites at establishment, parasites often lag behind during subsequent range expansion, and populations on the leading edge of the invasion may be exposed to a lower prevalence of parasites than longer established populations (Phillips et al. 2010). This may give invasion front populations an advantage and facilitate the growth of dense populations in newly colonised areas (Phillips et al. 2010).

Finally, if invasive species are susceptible to native parasites, acquisition of these parasites may impact the fitness of the invasive host (Krakau et al. 2006, Gendron et al. 2012, Paterson et al. 2013). In such cases, native parasites might affect the establishment, impact, or range expansion of an invasive species (Case and Taper 2000, Dunn 2009, Perkins 2012). Negative impacts of native parasites on invasive species have been recorded and likely affect the competitive ability of the invader (Krakau et al. 2006, Dunn 2009, Gendron et al. 2012); however, there is little empirical evidence of native parasites slowing range expansion or establishment.

Clearly, host-parasite dynamics can play a crucial role in both the success of an invasion and the impact the invader has on recipient communities. Thus, the fitness effects of parasitism, and the potential for transmission of parasites between invasive and native hosts, are important questions. Here we address these questions for an invasive gecko spreading into the gecko-rich woodlands of northern Australia.

The Asian house gecko, *Hemidactylus frenatus*, is a human-commensal species that has been accidentally introduced to many tropical and subtropical areas globally (Lever 2003; Hoskin 2011). *Hemidactylus frenatus* often establishes large populations where it is introduced, and in some areas it has caused declines and local extinctions of native species through competition (Cole et al. 2005, Dame and Petren 2006). Despite documented impacts in some areas, *H. frenatus* is typically considered a benign invader due to the belief that it is restricted to anthropogenic environments (Vanderduys and Kutt 2013). In northern Australia, however, *H. frenatus* are spreading from urban areas into natural environments (Hoskin 2011, Barnett et al. 2017). This range expansion is bringing *H. frenatus* into contact with a number of ecologically similar native species (Barnett et al. 2017). Are these native species acquiring the parasites of *H. frenatus*? Are *H. frenatus* acquiring native parasites? And, if so, do these native parasites affect body condition or survival of *H. frenatus*?

In this study, we focussed on mites and pentastomes of the genus *Waddycephalus* because they are visible externally on geckos in the field. At least seven species of *Geckobia* mites have been recorded on *H. frenatus* globally across its introduced range (Heath and Whitaker 2015), but only *Geckobia bataviensis* is previously known to have been introduced with *H. frenatus* into Australia (Domrow 1992; Hoskin 2011). There are many native gecko mites in Australia, including *Geckobia* and Trombiculid mites of the genera *Ascoschoengastia, Neotrombicula* and *Trombicula* (Domrow and Lester

1985). There are no records of *H. frenatus* hosting native Australian gecko mites, or of native Australian geckos hosting introduced mites, but this has not been assessed in any detail. *Geckobia* mites live their entire lives on geckos and are likely transmitted through direct contact between individuals (Bauer et al. 1990, Rivera et al. 2003), whereas the Trombiculid gecko mites have a free-living stage and geckos likely pick them up from their environment (Domrow and Lester 1985). The impacts of haematophagous mites on the health and fitness of geckos are largely unknown (Hanley et al. 1995).

Waddycephalus are endoparasitic pentastomes that have been detected in many Australian snakes (definitive hosts) and lizards (one of the intermediate hosts) (Riley & Self 1981; Riley et al. 1985; Barton 2007; Pare 2008; Kelehear et al. 2014b). Most species are described from Australia but the genus is also present in South-east Asia and Fiji (Riley & Self 1981). *Waddycephalus* have a complex multiple host lifecycle: adults parasitise the lungs of snakes, and the two intermediate hosts are likely coprophagous insects (e.g., cockroaches) and insectivorous lizards, frogs and small mammals (Riley & Self 1981; Pare 2008; Kelehear et al. 2014b). *Waddycephalus* nymphs encyst subcutaneously in geckos (Fig. 1A) and may excyst when the host is sick or stressed (Fig. 1B) (Paré 2008). Infection with nymphal pentastomes can significantly affect the host, with migration and moulting of nymphs being associated with host morbidity (Paré 2008). *Hemidactylus frenatus* have been recorded to host nymphs of one or more species of *Waddycephalus* in Australia (Barton 2007; Coates et al. 2017). These are assumed to be native *Waddycephalus* given the diversity and prevalence of the genus in Australian reptiles, and given that *Waddycephalus* infection is only seen in bushland populations of *H. frenatus* and not in urban populations (Barton 2007; Coates et al. 2016).

There has been one broad study of host-parasite dynamics in *H. frenatus* in Australia. Coates et al. (2017) assessed the prevalence of *Geckobia* mites (not identified to species), *Waddycephalus* (not identified to species) and *Raillietiella frenata*, an endoparasitic pentastomid known to have been introduced with *H. frenatus* into Australia (Barton 2007). They observed abrupt changes in parasite prevalence across the *H. frenatus* range edge (Coates et al. 2017). The prevalence of *Geckobia* mites declined past the urban edge, while *R. frenata* were completely absent outside of inner urban areas. In contrast, native *Waddycephalus* nymphs were found on *H. frenatus* in woodland environments and up to the urban edge (i.e., at the urban–woodland interface), but were absent in inner urban areas (Coates et al. 2017). The study concluded that during range expansion from urban to natural areas, *H. frenatus* may experience release from co-evolved parasites (*Geckobia* and *R. frenata*) but are exposed to novel native parasites (*Waddycephalus*). However, the study did not assess host specificity, detailed determinants of infection, and potential impacts of parasites on *H. frenatus* and native geckos.

In the current study we investigate host specificity of mites and *Waddycephalus* where invasive and native geckos co-occur in woodland habitats. We then investigate changes in parasite prevalence towards the invasion front of *H. frenatus*, and assess factors affecting individual infection probability and intensity. Finally, we investigate the effects of these parasites on *H. frenatus* body condition and draw inferences on impacts on survival.

Methods

The parasites

We focussed on mites and *Waddycephalus* nymphs in this study because they are visible externally on geckos in the field. Mites are visible as minute red or orange dots on the skin, often on the feet of geckos (Heath and Whitaker 2015), while *Waddycephalus* nymphs are visible as small protrusions under the skin of geckos (e.g., Fig 1A). We dissected three nymphs from one *H. frenatus* specimen to ensure that the protrusions under the skin were *Waddycephalus* nymphs. These nymphs were weighed in order to determine a representative weight to deduct in tests of host body condition.

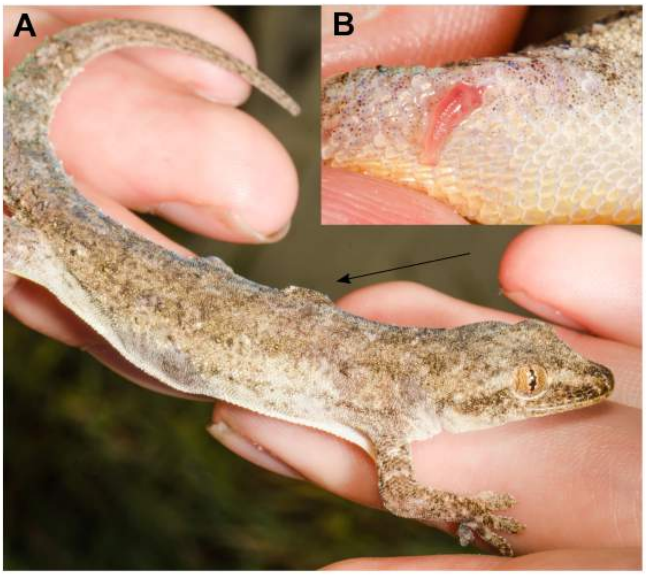


Figure 1 A. *Waddycephalus* nymphs encysted subcutaneously are visible as protrusions on the midbody of an adult *H. frenatus*, and **B.** a *Waddycephalus* nymph beginning to excyst after the gecko was captured. Photos: Matthew McIntosh.

Fieldwork

This study was conducted around the city of Townsville in north-east Australia, where *H. frenatus* have established large populations in dry sclerophyll woodland surrounding urban areas (Barnett et al. 2017). We used the same ten transects as Barnett *et al.* (2017). Each transect consisted of five survey sites, with Site 1 on the urban edge and subsequent sites 500 m apart out to Site 5 at 2 km (in a straight line) from the urban edge. The habitat at Site 1 was urban housing and gardens, and the other four sites were in adjacent woodland. Each site was surveyed for *H. frenatus* and native geckos once a month between May 2013 and April 2014, giving a total of 12 surveys per site, except for one site (Yabulu, YA) where ten surveys were conducted. We therefore conducted a total of 590 site surveys over 12 months. Sites on the same transect were surveyed on the same night but the order of surveying these sites was randomised for each visit.

At each site we conducted a five-minute listening survey, where we counted each time we heard the distinctive "chuck chuck chuck…" vocalisation of *H. frenatus,* and a 15-minute visual search, where we counted each *H. frenatus* or native gecko we found. During the 15-minute visual survey we walked slowly from a starting point, using head torches to locate geckos by their eye-shine. We also caught up to five *H. frenatus* on each survey to assess external parasites and to take measurements for body condition analyses. These geckos were placed inside a small snap-lock bag and weighed using a 10 g Pesola spring balance, and then measured for snout-vent length (SVL) using a small plastic ruler. Sex was determined by visually checking for testes bulges on males.

We began inspecting the captured *H. frenatus* for parasites during these surveys in September 2013 (the fifth month of surveys) and continued parasite screening through all surveys for the next eight months (a total of 400 surveys). Captured geckos were examined visually for *Waddycephalus* nymphs and mites. These mites were not collected for identification to species and therefore in this section we generalise all mites as '*Geckobia*'. The numbers of *Waddycephalus* nymphs and *Geckobia* mites on each gecko were counted. The 15-minute survey time was paused while geckos were being measured and examined for parasites. After examination, geckos were released at the point of capture.

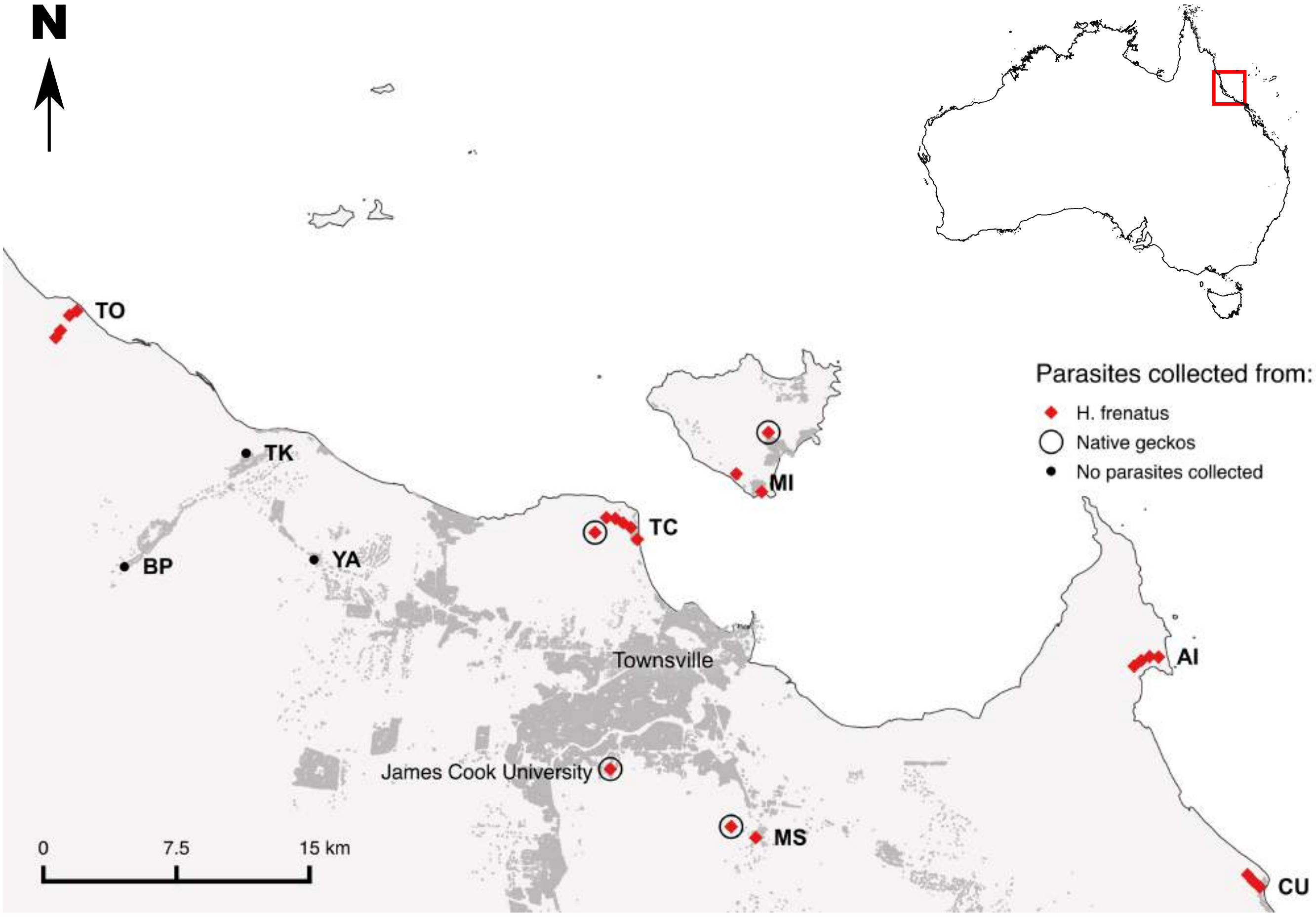


Figure 2. Map of the Townsville region, showing transects (two-letter codes) where parasite prevalence and intensity data for *H. frenatus* was collected. *Hemidactylus frenatus* and native geckos occur at all ten transects. Mites were collected for identification at a sub-set of these transects, from either *H. frenatus* (red diamonds) or native geckos (open circles). Additional mites were collected from geckos at a site close to the Magnetic Island (MI) transect and from the James Cook University campus. Buildings are shaded in dark grey.

Targeted Mite Collection

To assess the potential for host-switching we collected mites from *H. frenatus* (N = 95) and native geckos (N = 26). Mite collection was targeted to areas where native geckos and *H. frenatus* co-occur. This included sites at seven of the permanent transects as well as some nearby areas where *H. frenatus* and native geckos coexist (Fig. 2.). We also collected mites from geckos at the James Cook University campus (Fig. 2), an additional site where *H. frenatus* and native geckos co-occur on the urban–woodland interface. Mites were collected from the following native gecko species: *Gehyra dubia* (N = 20), *Amalosia rhombifer* (N = 4) and *Heteronotia binoei* (N = 2). All mites were identified morphologically to species by a specialist (AH).

Statistical analyses

All analyses were conducted in R version 3.1.2 (R Core Team 2016). All variables were scaled (sd=1) and mean centred prior to analyses to aid model fitting and to readily compare effect sizes. We used either linear mixed effects models (LMMs) or generalised linear mixed effects models (GLMMs), within the lme4 package (Bates et al. 2015), depending on the error structure of the response variable. To assess which factors affect parasite prevalence and the intensity of parasite infection, we analysed data for *Geckobia* mites and *Waddycephalus* nymphs in separate models (outlined below).

Which factors affect population-level parasite prevalence?

We used the first principle component of *H. frenatus* heard in listening surveys and *H. frenatus* seen in visual surveys as an estimation of relative abundance. These principle components were multiplied by -1 so that larger values represented higher relative abundance. Across sites, we found relative abundance and distance to the urban edge were not highly correlated (Spearman's rank correlation coefficient = -0.38), so we included both of these as independent variables in the following models.

We used GLMMs with binomial error structures and logit link functions to assess whether distance from the urban edge, relative abundance, or season affected 1. the number of infected/uninfected geckos with respect to *Geckobia* mites per survey, and 2. the number of infected/uninfected geckos with respect to *Waddycephalus* per survey. Seasonal changes were assessed by including Sine(day of year) and Cosine(day of year) as predictors in each model, where 'day of year' was transformed to fall between 0-2π (Stolwijk et al. 1999). Including seasonality in model in this way is useful because seasonal effects are fitted as a sinusoidal curve that fluctuates smoothly across the year—a better option than using as a categorical variable with limited biological significance, e.g. month of the year (Stolwijk et al. 1999). In both models we initially included the interaction between distance from the urban edge and relative abundance, but excluded this parameter in the final model if the interaction was not significant. Transect was included as a random effect in both models to account for broad variation in prevalence between transects.

Which factors affect infection probability and infection intensity on individual hosts?

GLMMs with binomial error structures were used to assess whether *H. frenatus* sex or body size (SVL) affected the likelihood of infection with 1. *Geckobia* mites or 2. *Waddycephalus* nymphs. In these analyses we were less interested in the across population and temporal effects, so we treated site and survey month as a random effects such that our fixed (individual-level) effects were conditioned on the mean prevalence at each site.time.

To assess whether sex or body size (SVL) of individuals affected the intensity of infection with *Geckobia* mites or *Waddycephlaus* nymphs we used zero-truncated GLMMs with negative binomial error distributions (to account for over dispersion). Zero-truncated distributions were necessary because we only included infected individuals in these analyses: intensity of infection could not equal zero. Site and survey month were included as random effects in both models

Do parasites affect body condition?

To investigate whether parasites affect body condition of *H. frenatus* we used LMMs with the natural log of gecko mass as the response variable. Here, we included *Waddycephalus* and *Geckobia* mites in the same models. First, we assessed whether the presence or absence of either parasite affected condition by including the following predictor variables: 1. the natural log of SVL, 2. the presence/absence of *Geckobia* mites, and 3. the presence/absence of *Waddycephalus*. We then investigated whether the intensity of infection affected body condition. In this model the predictor variables were: 1. the natural log of SVL, 2. the intensity of mite infection, and 3. the intensity of *Waddycephalus* infection. For individuals infected with *Waddycephalus*, gecko mass was first corrected by subtracting 0.005 g per nymph: the estimated mean weight of a nymph (calculated from weighing a subset of nymphs dissected out of a gecko). In both body condition analyses we included site and survey month as random effects.

Results

Co-occurrence and relative abundance of hosts

Hemidactylus frenatus was detected at 46 of the 50 transect sites over the survey period. *Hemidactylus frenatus* was most common at the urban edge sites but was present out to the furthest sites at 2 km from the urban edge on 9 out of 10 transects. Native geckos were detected at 47 of the 50 sites, including 43 of the sites where *H. frenatus* was detected. The mean number of *H. frenatus* was higher than native geckos at all distances from the urban edge (all transects combined; Fig. 3), and, summed for all the woodland sites (Sites 2–5), *H. frenatus* was much more common than all six native gecko species combined (Fig. 4).

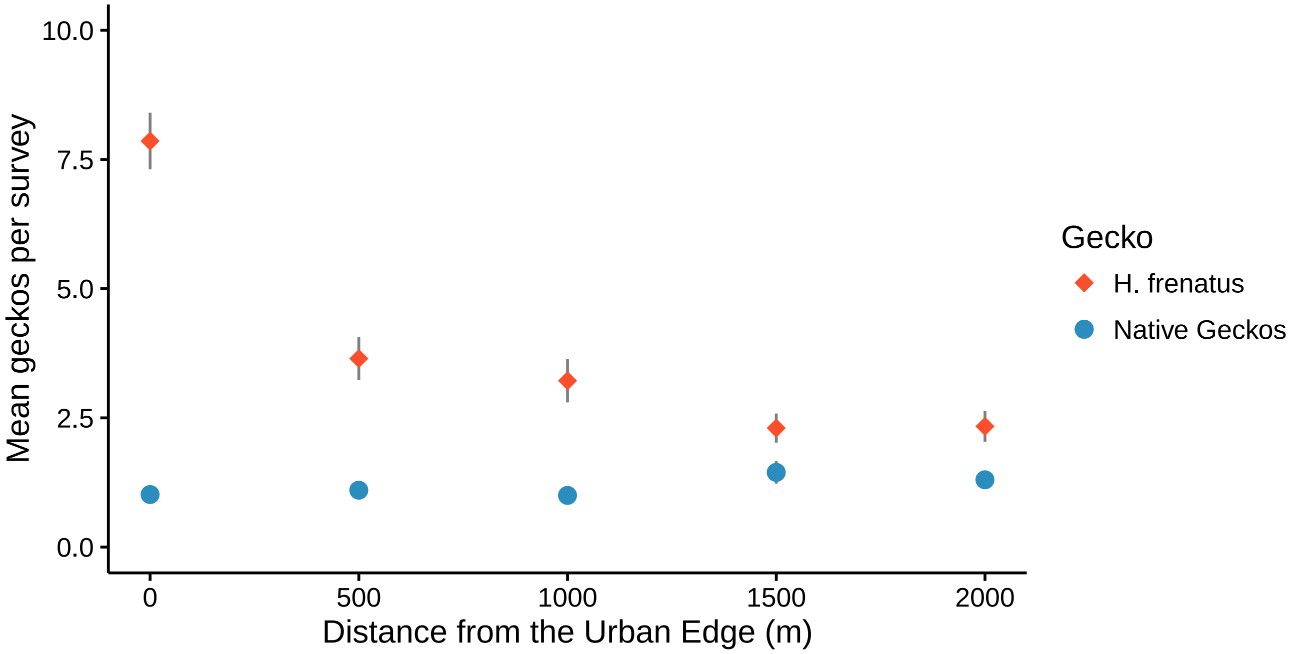


Figure 3. The mean number of *H. frenatus* and native geckos seen per survey, plotted for all transects combined.

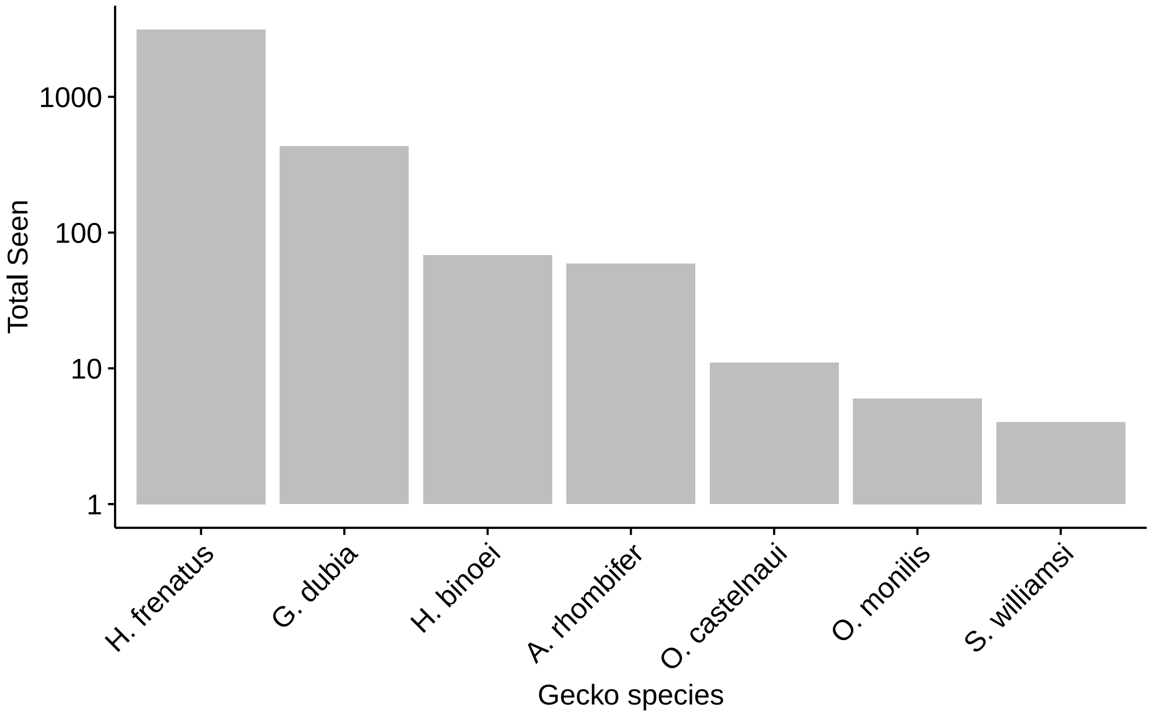


Figure 4. The total number of each gecko species seen at woodland sites (>500 m from the urban edge) throughout the 12-month survey period.

Host specificity

Targeted mite collections for identification revealed that *Hemidactylus frenatus* were infected exclusively with the introduced *Geckobia* mites, *G. bataviensis* and *G. keegani* (Table 1). This is the first record of *G. keegani* in Australia. Conversely, mites collected from three co-occurring native gecko species, *G. dubia, A. rhombifer* and *H. binoei,* were all native mite species (Table 1.). Both *G. dubia* and *A. rhombifer* are new host records for *G. gymnodactyli*. This is also the first record of *Amalosia rhombifer* hosting *Neotrombicula greenlyi*.

Waddycephalus nymphs were not identified to species so it was not possible to assess host specificity in detail. The nymph bulges were also observed on the native gecko

Gehyra dubia during the transect surveys, and have been observed on other reptiles locally (authors, pers. obs.). As covered in the Introduction, the *Waddycephalus* found in *H. frenatus* are deemed to be one or more native species, and hence infection of this introduced host is taken to represent host switching.

Table 1. *Geckobia* and *Neotrombicula* mites collected on *H. frenatus* and native geckos. *Geckobia sp.* in the final two columns refers to mites that could not be identified to species due to preservation condition.

							Geckobia sp.	
Gecko species	N	*G. bataviensis*	*G. keegani*	*G. bataviensis & G. keegani*	*G. gymnodactyli*	*Neotrombicula greenlyi*	Adults	Larvae
Hemidactylus frenatus	95	71	13	8	-	-	1	2
Gehyra dubia	20	-	-	-	17	-	3	-
Amalosia rhombifer	4	-	-	-	2	2	-	-
Heteronotia binoei	2	-	-	-	1	-	1	-

General patterns of parasite infection in H. frenatus

Mites were detected at high prevalence on *H. frenatus* on all transects (Table 2), and up to 2 km from the urban edge at five transects (TO, MI, MS, YA, TK). *Waddycephalus* nymphs were detected on *H. frenatus* on eight of the ten transects (Table 2.), at generally at low prevalence (Table 2). The maximum infection intensity for *Waddycephalus* was ten nymphs per gecko, observed in two individuals.

Table 2. Prevalence of parasites and mean intensity of infection in *H. frenatus* assessed during transect surveys in 2013–2014.

				Geckobia mites		*Waddycephalus* nymphs	
Transect	LAT	LONG	*H. frenatus* collected	Prevalence	Mean Intensity	Prevalence	Mean Intensity
Town Common (TC)	-19.20	146.77	108	0.37	13.18	0.07	3.13
AIMS (AI)	-19.27	147.05	88	0.30	10.92	0.06	1.20
Mount Stuart (MS)	-19.36	146.84	78	0.51	10.60	0.06	1.80
Cungulla (CU)	-19.39	147.11	69	0.48	12.36	0.03	2.00
Magnetic Island (MI)	-19.17	146.84	102	0.43	10.23	0.02	3.00
Toomulla (TO)	-19.08	146.47	75	0.47	10.66	0.09	4.14
Mount Elliot (ME)	-19.43	146.95	33	0.42	7.07	0.03	1.00
Yabulu (YA)	-19.13	146.36	28	0.43	9.75	0.14	3.25
Bluewater Park (BP)	-19.23	146.48	22	0.41	6.67	0.00	0.00
Toolakea (TK)	-19.159	146.56	10	0.60	10.33	0.00	0.00

Which factors affect population-level parasite prevalence in H. frenatus populations?

Distance from the urban edge significantly affected the prevalence of *Geckobia* mites in *H. frenatus* populations, with lower proportions of infected individuals found farther from the urban edge (P < 0.0001, t = -6.90, Fig. 5A, Table 3.). Relative abundance of *H. frenatus* did not affect the prevalence of mites directly (P = 0.59, t = - 0.54), but there was a positive interaction between distance and abundance, with a steeper cline in mite prevalence in relatively smaller host populations (P = 0.04, t = 2.05; Fig. 6). In Figure 6 we show how the interaction between distance from the urban edge and relative abundance affects *Geckobia* prevalence. Lines indicate *Geckobia* prevalence predicted from our model for different relative abundances including: the mean abundance of *H. frenatus* at our sites, low abundance (i.e., two standard deviations below the mean) and high abundance (i.e., two standard deviations above the mean).

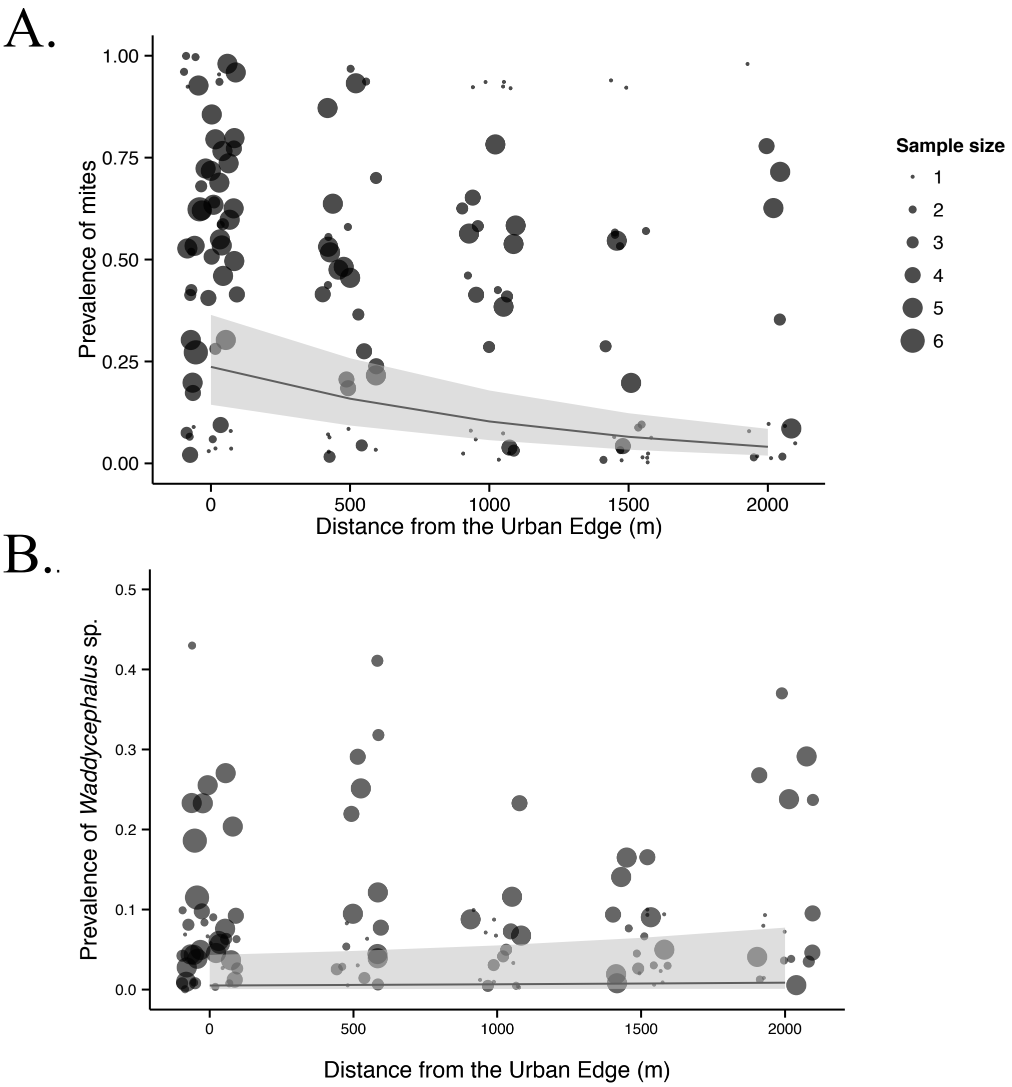


Figure 5. A. The prevalence of mite infection in *H. frenatus* per survey with distance from the urban edge. **B.** The prevalence of *Waddycephalus* sp. infection with distance from the urban edge. Size of the circles indicates sample size.

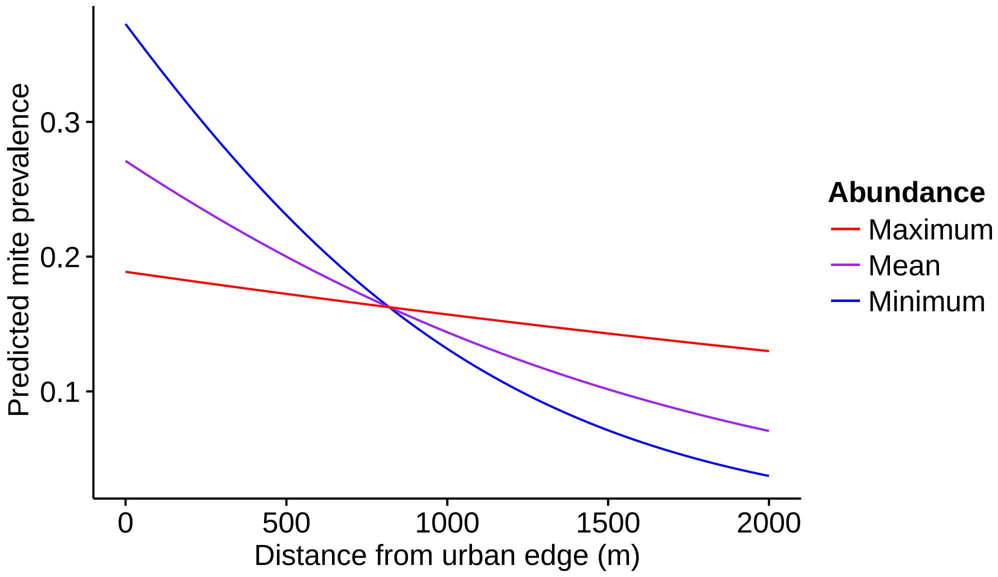


Figure 6. The interaction between distance from the urban edge and relative abundance on the prevalence of *Geckobia* mites predicted from our model. Maximum and minimum curves show expected values when abundance is two standard deviations above and below mean abundance respectively.

Table 3. Factors that affect prevalence of **A.** *Geckobia* mites, and **B.** *Waddycephalus* nymphs in *H. frenatus* populations

Model	Parameter	Estimate	Standard Error	t	P
A. Mite prevalence	Distance from urban edge***	-0.56	0.12	-4.53	< 0.0001
	Relative abundance	-0.05	0.10	0.54	0.59
	Sine(day)***	0.82	0.22	3.80	< 0.0001
	Cos(day)***	1.03	0.15	6.90	< 0.0001
	Distance.abundance*	0.20	0.10	2.05	0.04
B. Waddycephalus prevalence	Distance from urban edge	0.15	0.18	0.82	0.41
	Relative abundance	0.15	0.18	0.80	0.42
	Sine(day)*	1.60	0.76	2.11	0.03
	Cos(day)*	1.52	0.65	2.35	0.02

* P < 0.05
** P < 0.01
*** P < 0.0001

Prevalence of *Waddycephalus* nymphs was not affected by distance from the urban edge (P = 0.41, t = 0.82; Fig. 5B), or by relative abundance (P = 0.42, t= 0.80). Season significantly affected the prevalence of both mites (Sine(day): P < 0.0001, t = 3.88; Cosine(day): P < 0.0001 t = 6.95; Supp. Fig. 1A) and *Waddycephalus* (Sine(day): P = 0.03, t = 2.11; Cosine(day): P =0.02, z = 2.35; Supp. Fig. 1B, Table 3), with higher prevalence of parasites towards the middle of the year.

Which factors affect individual infection?

The likelihood of *Geckobia* mite infection (i.e. presence/absence of mites) was not significantly affected by either the sex (P = 0.94, t = 0.08) or SVL (P = 0.81, t = 0.24 Table 4) of geckos. Likelihood of infection with *Waddycephalus* sp. was similarly unaffected by the sex of individuals (P = 0.72, t = 0.36), but larger geckos were more likely to be infected with at least one *Waddycephalus* nymph (P < 0.01, t = 2.93, Table 4).

Table 4. Factors that affect the presence and infection intensity of *Geckobia* mites (models A and B), and *Waddycephalus* nymphs (models C and D) in individual *H. frenatus.*

Parasite	Model	Parameter	Estimate	Standard Error	t	P
Geckobia mites	**A.** Presence/Absence	Sex (male)	0.02	0.27	0.08	0.94
		SVL	0.05	0.19	0.24	0.81
	B. Infection Intensity (zero-truncated)	Sex (male)	0.04	0.16	0.27	0.79
		SVL**	0.28	0.10	2.83	< 0.01
Waddycephalus	**C.** Presence/Absence	Sex (male)	0.19	0.54	0.36	0.72
		SVL**	0.99	0.34	2.93	< 0.01
	D. Infection Intensity (zero-truncated)	Sex (male)	0.48	0.50	0.96	0.34
		SVL*	-0.56	0.27	-2.07	0.04

* P < 0.05
** P < 0.01

When looking only at infected individuals, sex did not affect the intensity of mite (P = 0.79, t = 0.27, Table 4), or *Waddycephalus* infection (P = 0.32, t = 1.00 , Table 4). Gecko size positively affected the intensity of mite infection (P < 0.01, t = 2.83, Table 4), but negatively affected the intensity of *Waddycephalus* infection, with smaller geckos having more *Waddycephalus* nymphs than larger geckos (P = 0.03, t = -2.12, Table 4.).

Do parasites affect body condition?

Body condition of geckos was not affected by infection with *Geckobia* (P = 0.15, t = -1.44) or *Waddycephalus* (P = 0.94, t = -0.07). There was also no significant effect of the intensity of *Geckobia* (P = 0.71, t = -0.37) or *Waddycephalus* infections on body condition (P = 0.29, t = 1.07).

Discussion

Host specificity and potential impact

We found large populations of *H. frenatus* out to the furthest sites 2 km from the urban edge on some transects. These populations were over three times larger than co-occurring native gecko species (Figs 3 & 4). This suggests that *H. frenatus* are achieving higher density in natural environments than are native geckos. The presence of comparatively large *H. frenatus* populations in natural environments gives the potential for both parasite spillover and spillback to be occurring in this system.

We found no evidence for spillover of mites—the invasive gecko had introduced mites and the native geckos had native mites (Table 1). This was surprising given the apparently ample opportunity for spillover. *Hemidactylus frenatus* co-occurs with native geckos at many of these sites, and is regularly found side-by-side with natives. Additionally, this invasive species has been present at some of these sites for many years (Barnett et al. 2017). Prevalence of mites on *H. frenatus* was high, and infected *H. frenatus* were present at most sites. The lack of evidence of host switching was unexpected because elsewhere in their introduced range, there are records of *H. frenatus* hosting *Geckobia* mites that are not present in their native range (Heath and Whitaker 2015). It was particularly unexpected given the relative abundance of *H. frenatus*– spillover from invasive to native species is predicted to be more common in areas where invasive hosts are higher density than native species (Kelehear et al. 2014b).

There are three potential reasons for the absence of mite transmission between invasive and native geckos in this system. First, *Geckobia* mites may rarely switch hosts due to their life history. Pterygosomatid mites spend their entire lives on geckos, and may only be transmitted during close and prolonged contact, such as mating or fighting (Bauer et al. 1990, Rivera et al. 2003). Indeed, Hanley et al. (1998) found no evidence of mite transfer between geckos (*Lepidodactylus* spp.) even after keeping them confined together for 48 hours (Hanley et al. 1995). While mites that have co-evolved with *H. frenatus* (i.e., *G. keegani* and *G. bataviensis*) have previously been recorded infecting other gecko species, including two members of the *Gehyra* genus (*G. oceanica and G. mutilata*), competency on these hosts has not been assessed (Heath and Whitaker 2015). Second, although *H. frenatus* and native geckos co-occur in many of the areas where we collected mites, they may generally occupy different microhabitats or actively avoid encounters with each other. Elsewhere in their invasive range *H. frenatus* exclude native *Nactus* geckos from retreat sites, resulting in fewer encounters between the two species (Cole et al. 2005). Third, it is possible that our mite sample size from native geckos (N = 26) was too small to detect transmission to native species. However, if that is the case, transmission frequency must be very low given the ample opportunity for transfer outlined above. Laboratory studies would further our understanding of host specificity of the introduced and native mites in this system.

We found *H. frenatus* infected with *Waddycephalus* nymphs on eight of the ten transects (Table 2), and we recorded *Waddycephalus* infections at all distances from the urban edge (Fig. 5B). Prevalence was generally low; a result echoed in the scarcity of *Waddycephalus* records from museum specimens (Barton 2007), but prevalence also appears low in native geckos in this system (CJH and LKB pers. obs.). Despite this low prevalence, the high abundance of *H. frenatus* may increase the prevalence of *Waddycephalus* in the final hosts (native snakes) and in turn other intermediate hosts (coprophagous insects and native geckos). We witnessed native snakes (*Boiga irregularis* and *Morelia spilota*) preying on *H. frenatus* on multiple occasions during the study. It seems likely, therefore, that *H. frenatus* provide the same trophic link as native geckos, enabling *Waddycephalus* to complete its life cycle and potentially increasing its prevalence in native hosts through parasite spillback.

Do parasites affect the range expansion of H. frenatus?

Prevalence of mites in *H. frenatus* populations decreased with distance from the urban edge. There was also a significant interaction between relative abundance and distance, with smaller populations having steeper declines in mite prevalence with distance from the urban edge (Fig. 6). This pattern of decreasing mite prevalence with distance from urban areas is consistent with Coates et al. (2017), who found the lowest prevalence of mites occurred 2 km into the woodland. There was, however, no detectible effect of mite presence on their host, in terms of impacts on body condition or survival.

There was also no relationship between the intensity of mite infection and body condition. Haematophagous mites can cause ulcerative dermatitis and inhibit skin sloughing in other lizards (Goldberg and Bursey 1991, Goldberg and Holshuh 1992, Walter and Shaw 2002), but to date there is no evidence of gecko mites affecting condition in wild populations (Hanley et al. 1995). When looking at only individuals infected with mites, we found significantly more mites on larger geckos. If mites affected the lifespan of geckos one would expect to see fewer highly infected large (i.e., older) individuals. These results suggest that the impact of mites on host survival may be negligible. It is, therefore, unlikely that release from mites impacts range expansion in this system, despite an apparently lower prevalence of mites on the invasion front.

The prevalence of *Waddycephalus* was not affected by distance from the urban edge or relative abundance of *H. frenatus*. Predictions regarding prevalence of *Waddycephalus* in *H. frenatus* are complicated by the fact that *Waddycephalus* have a complex lifecycle, and so are limited by the abundance of their final hosts (native snakes) and their first intermediate hosts (likely coprophagous insects) (Ali and Riley 1983, Kelehear et al. 2014a). The *Waddycephalus* in *H. frenatus* are most likely a native species (or multiple native species) and *H. frenatus* are infected at low prevalence at most of our woodland sites. In contrast, the parasite has been found to be completely absent in adjacent inner urban sites, probably due to smaller snake populations (Coates et al. 2017).

Due to their presence in natural areas but absence in inner urban environments, *Waddycephalus* could potentially affect range expansion of *H. frenatus* in natural habitats if infection impacts body condition or survival. We found no effect of presence or intensity of *Waddycephalus* nymphs on body condition of *H. frenatus*. In infected individuals, however, smaller geckos had a greater intensity of infection than larger geckos (Table 4). One explanation of this pattern is that high intensity *Waddycephalus* infection increases mortality with time, so adult geckos with high intensity infection are missing from the population. While data on the effects of nymphal pentastomes on *H. frenatus* is lacking, they can kill reptile hosts when they migrate or moult (Paré 2008), particularly when they burrow from the stomach to the body wall (Paré 2008), or affect the likelihood of predation through changes to host behaviour (Lefèvre et al. 2009). Another explanation is that some nymphs are lost through time, either because they excyst or because adult geckos have some way of shedding them. Future studies should further explore the impact of *Waddycephalus* nymphs on geckos.

Conclusion

This study examined the potential impacts of an invasive gecko on native species through parasite transmission, and the potential effect of parasites on range expansion of the invasive gecko. We found no evidence of invasive *Geckobia* mites infecting native geckos in this system, or *vice versa*, despite apparently ample opportunity in terms of time and fine scale co-occurrence. *Hemidactylus frenatus* are, however, susceptible to infection by native *Waddycephalus* nymphs, and high intensity infections may reduce the survival of individual *H. frenatus*. We explored the complex interactions between parasites and range expansion of this invasive gecko species and found that range expansion into natural environments means both release from co-evolved mites and exposure to novel *Waddycephalus* nymphs. The relatively high density of *H. frenatus* makes parasite spillback (of *Waddycephalus*) to native host species a concern. Future work should investigate parasite spillback by assessing whether prevalence of *Waddycephalus* nymphs in native gecko and snake populations is higher where they co-occur with *H. frenatus*. Detailed research is required to determine the impact of *Geckobia* mites on their hosts. While they are typically assumed to be parasites, the deeply invaginated armpits on many geckos and other lizard hosts (e.g., skinks), often filled with mites, suggests a potentially beneficial relationship.

References

Ali, J. H. and Riley, J. 1983. Experimental life-cycle studies of Raillietiella gehyrae Bovien, 1927 and Raillietiella frenatus Ali, Riley and Self, 1981: pentastomid parasites of geckos utilizing insects as intermediate hosts. - Parasitology 86: 147–160.

Andreou, D. et al. 2012. Introduced pathogens and native freshwater biodiversity: a case study of Sphaerothecum destruens. - PLoS One 7: e36998.

Barnett, L. K. et al. 2017. Going feral: Time and propagule pressure determine range expansion of Asian house geckos into natural environments. - Austral Ecol. 42: 165–175.

Barton, D. P. 2007. Pentastomid parasites of the introduced Asian house gecko, Hemidactylus frenatus (Gekkonidae), in Australia. - Comp. Parasitol. 74: 254–259.

Bates, D. et al. 2015. Fitting linear mixed-effects models using lme4. - J. Stat. Softw. 67: 1–48.

Bauer, A. M. et al. 1990. Skin folds in the gekkonid lizard genus Rhacodactylus: a natural test of the damage limitation hypothesis of mite pocket function. - Can. J. Zool. 68: 1196–1201.

Case, T. J. and Taper, M. L. 2000. Competition, environmental gradients, gene flow and the coevolution of species' borders. - Am. Nat. 155: 583–605.

Coates, A. et al. 2017. Living on the Edge: Parasite Prevalence Changes Dramatically across a Range Edge in an Invasive Gecko. - Am. Nat. in press.

Cole, N. C. et al. 2005. The need for enemy-free space: the impact of an invasive gecko on island endemics. - Biol. Conserv. 125: 467–474.

Dame, E. A. and Petren, K. 2006. Behavioural mechanisms of invasion and displacement in Pacific island geckos (Hemidactylus). - Anim. Behav. 71: 1165–

1173.

Domrow, R. 1992. Acari Astigmata (excluding feather mites) parasitic on Australian vertebrates: an annotated checklist, keys and bibliography. - Invertebr. Syst. 6: 1459.

Domrow, R. and Lester, L. N. 1985. Chiggers of Australia (Acari: Trombiculidae): an annotated checklist, keys and bibliograpy. - Aust. J. Zool. 114: 1–111.

Dunn, A. M. 2009. Parasites and biological invasions. - Elsevier Ltd.

Dunn, A. M. and Hatcher, M. J. 2015. Parasites and biological invasions: parallels, interactions, and control. - Trends Parasitol. 31: 189–199.

Dunn, A. M. et al. 2012. Indirect effects of parasites in invasions. - Funct. Ecol. 26: 1262–1274.

Floerl, O. et al. 2009. The importance of transport hubs in stepping-stone invasions. - J. Appl. Ecol. 46: 37–45.

Gendron, A. D. et al. 2012. Invasive species are less parasitized than native competitors, but for how long? The case of the round goby in the Great Lakes-St. Lawrence Basin. - Biol. Invasions 14: 367–384.

Goldberg, S. R. and Bursey, C. R. 1991. Integumental lesions caused by ectoparasites in a wild population of the side-blotched lizard (Uta stansburiana). - J. Wildl. Dis. 27: 68–73.

Goldberg, S. R. and Holshuh, H. J. 1992. Ectoparasite-induced lesions in mite pockets of the Yarrow’s spiny lizard, Sceloporus jarrovii (Phrynosomatidae). - J. Wildl. Dis. 28: 537–541.

Hanley, K. A. et al. 1995. Lower mite infestations in an asexual gecko compared with its sexual ancestors. - Evolution (N. Y). 49: 418–426.

Hartigan, A. et al. 2011. A suspected parasite spill-back of two novel Myxidium spp.

(Myxosporea) causing disease in Australian endemic frogs found in the invasive Cane toad. - PLoS One 6: e18871.

Heath, A. C. G. and Whitaker, A. H. 2015. Mites (Acari: Pterygosomatidae, Macronyssidae) taken from lizards intercepted at the New Zealand border. - Syst. Appl. Acarol. 20: 739.

Heredia, V. J. et al. 2014. Mites in the neotropical lizard Liolaemus pacha (Iguania: Liolaemidae): relation to body size, sex and season. - South Am. J. Herpetol. 9: 14–19.

Hoskin, C. J. 2011. The invasion and potential impact of the Asian House Gecko (Hemidactylus frenatus) in Australia. - Austral Ecol. 36: 240–251.

Hudson, P. and Greenman, J. 1998. Competition mediated by parasites: biological and theoretical progress. - Trends Ecol. Evol. 13: 387–390.

Kelehear, C. et al. 2013. Invasive parasites in multiple invasive hosts: the arrival of a new host revives a stalled prior parasite invasion. - Oikos: no-no.

Kelehear, C. et al. 2014a. Pentastomids of wild snakes in the Australian tropics. - Int. J. Parasitol. Parasites Wildl. 3: 20–31.

Kelehear, C. et al. 2014b. An introduced pentastomid parasite (Raillietiella frenata) infects native cane toads (Rhinella marina) in Panama. - Parasitology 142: 675–679.

Kelly, D. W. et al. 2009. Parasite spillback: a neglected concept in invasion ecology. - Ecology 90: 2047–2056.

Krakau, M. et al. 2006. Native parasites adopt introduced bivalves of the North Sea. - Biol. Invasions 8: 919–925.

Lefèvre, T. et al. 2009. The ecological significance of manipulative parasites. - Trends Ecol. Evol. 24: 41–48.

Lever, C. 2003. Naturalized reptiles and amphibians of the world. - Oxford University Press.

Menge, B. A. 1972. Competition for food between two intertidal starfish species and its effect on body size and feeding. - Ecology 53: 635–644.

O’Dowd, D. J. et al. 2003. Invasional “meltdown” on an oceanic island. - Ecol. Lett. 6: 812–817.

Paré, J. a. 2008. An overview of pentastomiasis in reptiles and other vertebrates. - J. Exot. Pet Med. 17: 285–294.

Paterson, R. A. et al. 2013. Native fish avoid parasite spillback from multiple exotic hosts: Consequences of host density and parasite competency. - Biol. Invasions 15: 2205–2218.

Perkins, T. A. 2012. Evolutionarily labile species interactions and spatial spread of invasive species. - Am. Nat. 179: E37–E54.

Phillips, B. L. et al. 2010. Parasites and pathogens lag behind their host during periods of host range advance. - Ecology 91: 872–81.

Prenter, J. et al. 2004. Roles of parasites in animal invasions. - Trends Ecol. Evol. 19: 385–90.

R Core Team 2016. R: A language and environment for statistical computing.: R Foundation for Statistical Computing, Vienna, Au.

Rivera, C. C. M. et al. 2003. Hemidactylus mabouia (Sauria: Gekkonidae), host of Geckobia hemidactyli (Actinedida: Pterygosomatidae), throughout the Caribbean and South America. - Caribb. J. Sci. 39: 321–326.

Stolwijk, A. M. et al. 1999. Studying seasonality by using sine and cosine functions in regression analysis. - J. Epidemiol. Community Health 53: 235–238.

Torchin, M. E. and Mitchell, C. E. 2004. Parasites, pathogens, and invasions by plants and animals. - Front. Ecol. Environ. 2: 183–190.

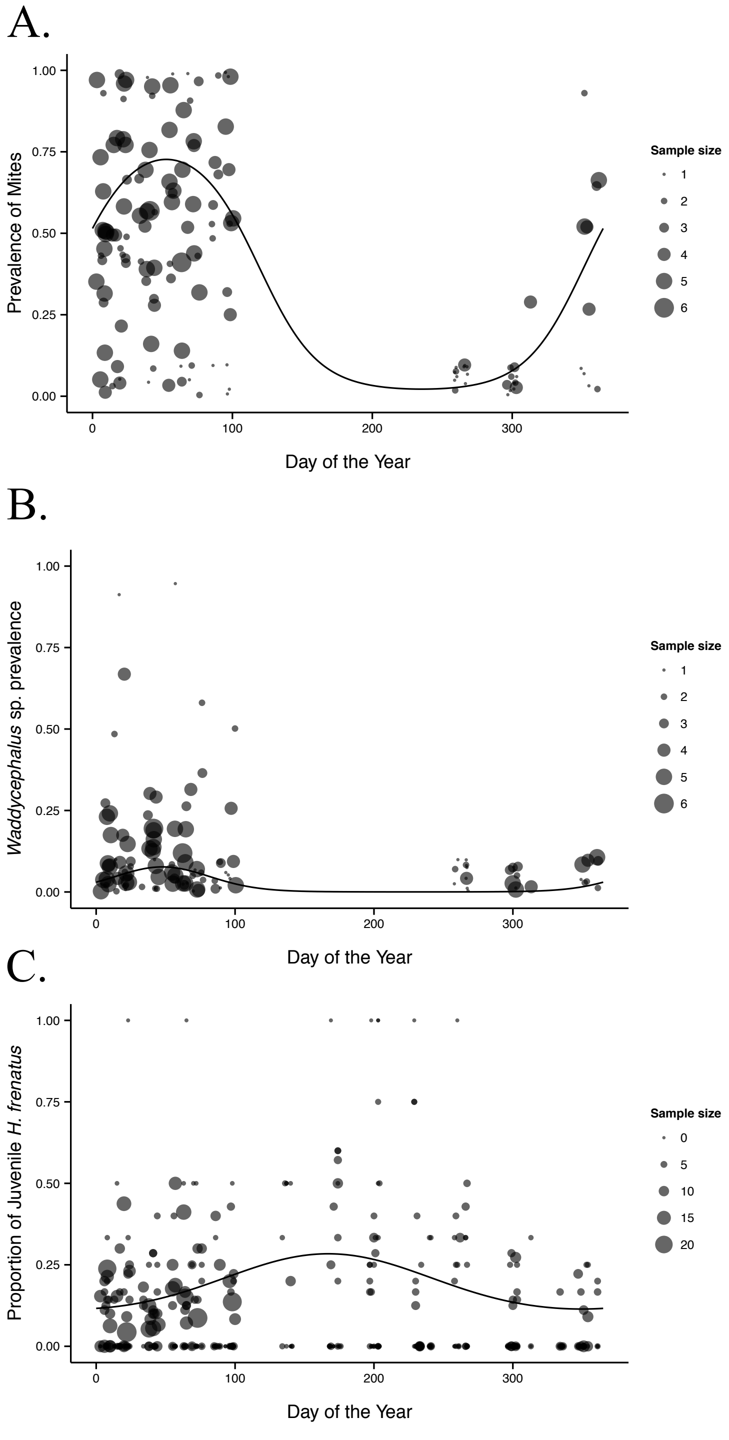
Torchin, M. E. et al. 2003. Introduced species and their missing parasites. - Nature 421: 628–630.

Vanderduys, E. P. and Kutt, A. S. 2013. Is the Asian house gecko, Hemidactylus frenatus, really a threat to Australia’s biodiversity? - Aust. J. Zool. 60: 361–367.

Walter, D. E. and Shaw, M. 2002. First record of the mite Hirstiella diolii Baker (Prostigmata: Pterygosomatidae) from Australia, with a review of mites found on Australian lizards. - Aust. J. Entomol. 41: 30–34.

Wanless, R. M. et al. 2007. Can predation by invasive mice drive seabird extinctions? - Biol. Lett. 3: 241–244.

Supplementary Material



Supplementary Figure 1. A. The effect of season on the prevalence of *Geckobia* sp. infection and B. *Waddycephalus* infection in *H. frenatus* populations. As well as C., patterns of juvenile recruitment in these populations (latter taken from Barnett et al. 2017).

Chapter 5.

Modeling individual-level detection probability of invasive Asian house geckos in natural environments

---

Louise K Barnett[1], Conrad J Hoskin[1] and Ben L Phillips[2]

[1]*Centre for Tropical Biodiversity & Climate Change, College of Science & Engineering, James Cook University, Townsville, Queensland 4811, Australia.*

[2]*School of BioSciences, University of Melbourne, Parkville, Victoria 3010, Australia.*

17 March 2017

Manuscript for consideration in: *Biological Invasions*

Abstract

Imperfect detection presents a major challenge in understanding and controlling the spread of invasive species. Effective surveillance and eradication require the early detection of individual invaders. Most studies of detection, however, focus on population-level detection probability (i.e., the likelihood of detecting at least one individual in a population). Here, we use binomial mixture models to assess individual-level detection probability (i.e., the probability of detecting a single individual, regardless of population size) in a pantropical invasive species (the Asian house gecko, *Hemidactylus frenatus*). Data were collected during 10-12 repeat surveys over 12 months at 50 sites in north-eastern, Australia. We assessed the abiotic factors affecting individual-level detection, and compared the efficiency of listening to visual surveys across a range of conditions. We found that – in both listening and visual surveys – season, temperature, time of night and the presence of wind affected *H. frenatus* detection. Observer experience, however, was only important for visual surveys. While multiple site visits are necessary for reliable detection of individual *H. frenatus*, surveying in appropriate conditions (i.e., soon after sunset on warm summer nights with no wind) can result in a five-fold decrease in the number of surveys needed for reliable detection. Thus, characterising the correlates of individual-level detection is a powerful means for developing optimal survey methods, and for setting baseline levels of effort required for adequate surveillance.

Key words: Detection; survey effort; *Hemidactylus frenatus;* seasonal; invasive

Introduction

To develop efficient monitoring and surveillance programs programmes for invasive species, it is vital to understand the factors that affect detection (Conway et al. 2008, Christy et al. 2010). Early, reliable detection is critical for quarantine, surveillance, and eradication efforts, and also for estimating population size (Dejean et al. 2012). Without reliable detection, invasive populations may go unnoticed for many years after establishment (Tingley et al. 2014), or invasions that were thought to be under control may flare up again from undetected populations (Keith and Spring 2013). Reliable detection is, however, challenging. Populations may not be obvious until they reach a threshold size, which may be too late for effective management (Britton et al. 2011, Dejean et al. 2012). Some species (particularly small, cryptic taxa) are intrinsically difficult to detect, and habitat complexity can exacerbate this problem (Green et al. 2013; Somaweera et al. 2010). Detection will also often vary through time due to seasonal and abiotic drivers. Thus, reliable detection can be challenging, and imperfect detection presents a major challenge in understanding and controlling the spread of invasive species (Metson et al. 2012).

It is important to distinguish between two levels of detection probability for surveillance and monitoring of invasive populations. The first is population-level detection, which is the probability of detecting the presence of an invasive population (MacKenzie et al. 2002). Population-level detection is a function of population size, with large populations being substantially easier to detect (Pellet and Schmidt 2005). The second level is individual-level detection, or the probability of detecting a single invasive organism, regardless of population size (Christy et al. 2010). Individual-level detection will be affected by abiotic factors, such as time of day, temperature, precipitation, moon phase and wind speed (Tang et al. 2001, Conway et al. 2008, Dejean et al. 2012), and can also vary between observers (Boulinier et al. 1998).

While it is increasingly common to account for imperfect detection when assessing the presence or absence of a species, these studies rarely identify the relationship between time-varying environmental conditions and detection probability (MacKenzie et al. 2002, Wenger and Freeman 2008). This is because most studies of detection focus on population-level detection (Royle and Nichols 2003, Mackenzie 2005, Gormley et al. 2011). For invasive species, however, it is arguably more important to understand individual-level detection probability, because effective surveillance and eradication require the early detection of individual invaders (Christy et al. 2010). Here, we estimate individual-level detection for a pantropical invasive species (the Asian house gecko), and show how detection is affected by conditions on the day of the survey as well as the time of year. We demonstrate how this information can then be used to estimate population size and also to develop optimal survey design for detection of this species.

The Asian house gecko (*Hemidactylus frenatus)*

The Asian house gecko, *Hemidactylus frenatus* is native to south and South-east Asia (Case et al. 1994, Lever 2003, Hoskin 2011) but is currently one of the world’s most widespread terrestrial reptiles, having been accidentally introduced to many tropical and subtropical areas around the world (Hoskin 2011). The current range of *H. frenatus* includes Australia, Japan, parts of South-East Asia, central America, Mexico, the southern United States, Kenya, Madagascar, and island nations in the Indian, Pacific and Atlantic oceans (Lever 2003, Rödder et al. 2008, Hoskin 2011).

Hemidactylus frenatus excel at human-mediated jump dispersal. In fact, a recent study found that *H. frenatus* contributed to 44 % of all reptile and amphibian quarantine interceptions in New Zealand (Chapple et al. 2016). The following characteristics of *H. frenatus* contribute to invasion success: 1. it has strong associations with humans and is often found foraging for insects around lights on buildings (Hoskin 2011; Zozaya et al. 2015), 2. it is a small species (max snout-vent length = 60 mm) and easily goes unnoticed during transportation (Hoskin 2011), 3. female *H. frenatus* can store sperm for up to 12 months (Yamamoto and Ota 2006), and 4. eggs of *H. frenatus* are hard-shelled so are desiccation resistant and can even tolerate exposure to seawater (Hoskin 2011).

Upon introduction, *H. frenatus* readily establish large populations and can outcompete native species in urban areas, as well as in natural habitat on oceanic islands (Cole et al. 2005, Hoskin 2011). While colonisation of natural habitats has rarely been documented across the introduced range (with the exception of islands) (Hoskin 2011), in parts of northern Australia, *H. frenatus* have established large self-sustaining populations in natural environments (Barnett et al. 2017). The extent of this invasion from urban areas into woodland is determined by time and propagule pressure (i.e., size of the urban population) (Barnett et al. 2017), suggesting further invasion of natural habitats is likely in northern Australian and elsewhere. This reliance on propagule pressure also means that if small populations of *H. frenatus* are detected early enough, effective removal may be possible in areas of conservation concern. It is, therefore, vital to understand the factors that control detection probability in this habitat in order to accurately monitor arrival and spread of this species.

Detection of *H. frenatus* is likely strongly controlled by abiotic factors. Like all geckos, *H. frenatus* are ectotherms and rely on favourable thermal conditions to forage (Lei and Booth 2014). They are also small, cryptic and nocturnal, and therefore populations may initially be difficult to detect, especially in complex natural habitats. In Australia, *H. frenatus* are often mistaken for visually similar native species (indeed for several years one of the major species distribution websites —the Atlas of Living Australia™— featured pictures of *H. frenatus* under the profiles of two native Australian geckos, *Gehyra australis* and *G. dubia,* due to visual similarities (Supp. Fig. 1). Spines on either side of *H. frenatus* tails are commonly used to distinguish them from native *Gehyra* geckos, which do not have spines. However, these spines are absent on *H. frenatus* that have regenerated tails and are present in some native species, leading to potential confusion of untrained observers.

Hemidactylus frenatus use loud vocalisations to communicate and these distinctive calls can be used to identify the species. The ‘multiple chirp’ or ‘chuck, chuck, chuck…’ call of the species is thought to be used in aggressive interactions between males (Marcellini 1977), but call function remains poorly resolved. Loud vocalization is rare in reptiles, and in Australia *H. frenatus* is one of the few geckos known to commonly vocalize. Automated sound recording devices are currently being used in key areas (e.g., ports and off-shore conservation islands) to attempt early detection of *H. frenatus*. To date, however, there has been no investigation into the factors affecting auditory detection of this species.

The aim of this study is to develop a method for accurately detecting *H. frenatus* in natural environments through: 1) assessing which abiotic factors influence visual and auditory detection of individual *H. frenatus* and, 2) estimating the number of surveys needed for accurate detection of this species in a variety of environmental conditions.

Methods

Gecko Surveys

This study was conducted in the Townsville region of north Queensland where *H. frenatus* have established populations in natural environments. We surveyed the same ten transects as Barnett *et al.* (2016). Each transect was made up of five sites (so 50 sites total), each positioned every 500 m from the urban edge up to 2 km (in a straight line) into surrounding woodland. We surveyed each transect once a month for a year (12 times) between May 2013 and April 2014, except for one transect, which was only surveyed ten times. This resulted in a total of 590 site surveys over 12 months.

Each survey was 20 minutes in duration and consisted of a 5-minute listening survey at the site marker, followed by a 15-minute visual search. For the 5-minute listening survey, two surveyors stood silently and counted the number of distinctive multiple chirp (“chuck chuck chuck…”) calls of *H. frenatus* heard over a five-minute period. For the 15-minute visual survey, the surveyors then slowly walked in a straight line from the listening point and used head torches to locate *H. frenatus* by eye-shine. Sites on the same transect were surveyed on the same night, but the order in which sites were surveyed was randomised on each visit. At the start of each survey we recorded the time, temperature, and wind (wind in the Townsville area falls into two states: light wind or still).

Louise Barnett and a volunteer research assistant conducted all surveys. Research assistants varied between survey nights, but were all trained in the detection techniques prior to commencement of the survey. The experience level of each research assistant was ranked on a scale of 1 (no previous experience in using eye-shine to detect animals) through to 10 (highly experienced in using eye-shine to locate small reptiles). For each additional transect surveyed by a research assistant (i.e., 5 sites), their experience score improved by 1 until a maximum experience score of 10 was achieved. Combined surveyor experience was calculated by taking the average experience level of the volunteer and the primary researcher (LKB).

Data Preparation

For each survey night we downloaded moonrise, moonset and moon illumination data for Townsville from the website ‘timeanddate.com’. We then generated a variable for ‘moon illumination’, by firstly assessing whether the moon had risen at the time of the survey. If so, we used the percentage of moon illumination from timeanddate.com. If not, the moon illumination value was set to zero.

To assess the effects of season on detection probability we transformed the day of year on which each survey was conducted into radians ($Day\ of\ year \times \frac{2\pi}{365}$). This value was then expressed as the sine and cosine, which allowed us to model changes in detection probability over time as a sinusoidal curve across the year (Stolwijk et al. 1999, Bruemmer et al. 2010).

Analyses

To investigate whether the number of *H. frenatus* vocalisations heard during listening surveys was correlated with the number of *H. frenatus* sighted in visual surveys we used a Generalised Linear Mixed Effects Model (GLMM) with a negative binomial error distribution (to account for over dispersion). In this model survey number (1-12) was included as a random effect to account for seasonal variation.

To investigate which factors affected *H. frenatus* detection, we used two binomial mixture models (a model for listening surveys and a model for visual surveys). Each model allowed abundance to vary between sites across each of the 50 survey sites (Royle 2004, Oppel et al. 2014). The models were fitted in a Bayesian framework, using JAGS (Just Another Gibbs Sampler), which we ran within R (R Core Team 2016) through the ‘rjags’ package (Plummer 2016).

In the following models N_i is defined as the abundance of individuals within each survey area, i.e. the number of geckos you would see or hear if every individual in the survey area was perfectly detectable. The models assume a closed population within the survey area and a constant value for Ni throughout the year. This assumption is necessary to make the problem tractable within our logistical constraints. Invariant population size within a year is not likely to be strictly true in nature, but is likely reasonable compared to inter-annual variability in population size. It is also important to note that the survey area was different for visual and listening surveys. In listening surveys the observers stood in one spot and may have heard *H. frenatus* within a radius of approximately 50 m in ideal conditions. During visual surveys, however, surveyors walked approximately 200 m, spotting geckos up to approximately 15 m off the path, depending on habitat. Because of the likely difference in effective survey area, we modelled visual and listening surveys separately.

Observation model:

Our model consists of an observation process describing the number of individuals seen (or heard) during each survey. We assumed that the observed number of individuals was drawn from a binomial distribution parameterised with the true number of individuals within the survey area (constant across time, but varying across sites N_i), and the probability of detecting each of these individuals (constant across individuals in each survey, but varying through time, p_t).

$$N_{obs} \sim Binomial(p_t, N_i)$$

Abundance model:

We then made each of these parameters a function of covariates using linear models with appropriate link functions. The true number of individuals at a site was treated as a Poisson regression (with log link) with time since *H. frenatus* colonisation, distance of each site from the urban edge, and the interaction of these two factors as predictors. These were included because in a previous study the interaction between these two factors significantly affected relative abundance of *H. frenatus* (Barnett et al., 2016). Transect was also included as a random effect to account for broad differences in abundance between locations.

$$N_i \sim Poiss(\lambda_i)$$

$$\log(\lambda_i) \propto distance_i \times colonisation\ time + transect$$

Detection model:

We treated the detection probability, p_t, also as a linear function (with a logit link). Predictor variables in this case were time dependent: 1. temperature, 2. time of survey (minutes post sunset), 3. moon illumination (0–100 %), 4. wind (present or absent), 5. observer experience, and 6. Season: cos (day of year) + sin(day of year) where day of year was transformed to vary between 0 and 360.

$$logit(p_t) \propto temp. + time + moon\ illum. + wind + obs.exp. + season$$

For each analysis we used minimally informative priors (see Supp. Table 1.) and ran three Markov chains for 650,000 iterations. We discarded the first 250,000 iterations as a burn-in and thinned the remaining data by five. Model convergence was assessed using trace plots, as well as the Gelman-Ruben diagnostic test (Toft et al. 2007), which revealed acceptable levels of convergence for all parameters, with values close to one.

In order to compare efficiency of listening and visual surveys, detection probability for five-minute visual surveys was calculated post hoc using the following transformation of 15-minute visual detection probability:

$$p_{5min} = 1 - (1 - p_{15min})^{0.33}$$

Scaling detection probability over multiple surveys

Assuming surveys are independent with respect to individual-level detection probability, we can easily observe the effect of multiple surveys on the cumulative individual-level detection probability using the relationship: *C(n)* = $1 - (1 - p_t)^n$.

This describes how individual-level detection scales with the number of surveys (*n*), which is a useful means for comparing surveillance effort.

Results

We conducted 590 surveys (each consisting of both a listening and a visual survey) over 12 months. We detected *H. frenatus* on 73% of these surveys. On 46% of surveys we detected *H. frenatus* in both listening and visual surveys (Fig. 1); on 21% of surveys we detected *H. frenatus* during visual surveys but not listening surveys; and on 6% of surveys *H. frenatus* were detected during listening surveys but not visual surveys. There was a weak but positive relationship between the number of geckos seen in visual surveys and the number of geckos heard in listening surveys (z = 10.94, P < 0.001, Fig. 1).

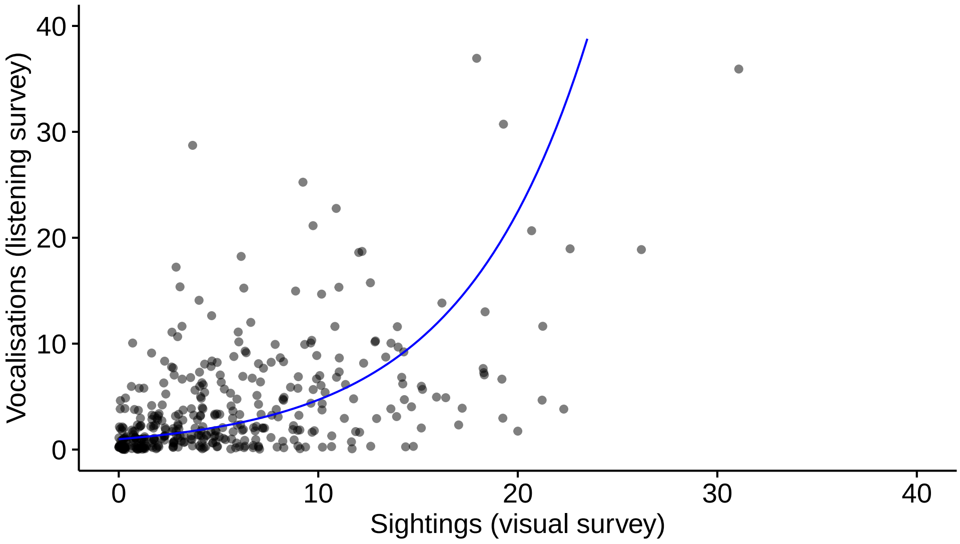


Figure 1. Number of sightings and vocalisations recorded for *H. frenatus* per site visit over the 12 months of sampling. Each dot is a 20 minute survey: the number of calls heard in the 5 minute listen versus the number of geckos seen in the 15 minute search component. The line shows the predicted number of vocalisations derived from our model.

Correlates of abundance

Distance from the urban edge negatively affected the estimated abundance of *H. frenatus* at each site (Table 1). There was also a positive interaction between distance and time since colonisation, with larger populations of *H. frenatus* more likely to be found further from the urban edge on transects where *H. frenatus* has had a longer establishment history in the adjacent urban area.

Correlates of individual-level detection

Listening surveys

Season had a large effect on individual-level detection probability for *H. frenatus* in listening surveys (Fig. 2), with detection more likely in summer. In addition to this seasonal effect, detection probability in listening surveys was positively affected by increased temperature: over the range of temperatures we surveyed, temperature differences resulted in a three-fold change in detection probability in listening surveys (Fig. 3A). Increasing time after sunset (Fig. 3B) and the presence of wind (Fig. 3D) decreased detection probability in listening surveys (Fig. 3; Table 1). By contrast, moon illumination and observer experience (Fig. 3C) had negligible effects on auditory detection of *H. frenatus*, with credible intervals here overlapping zero.

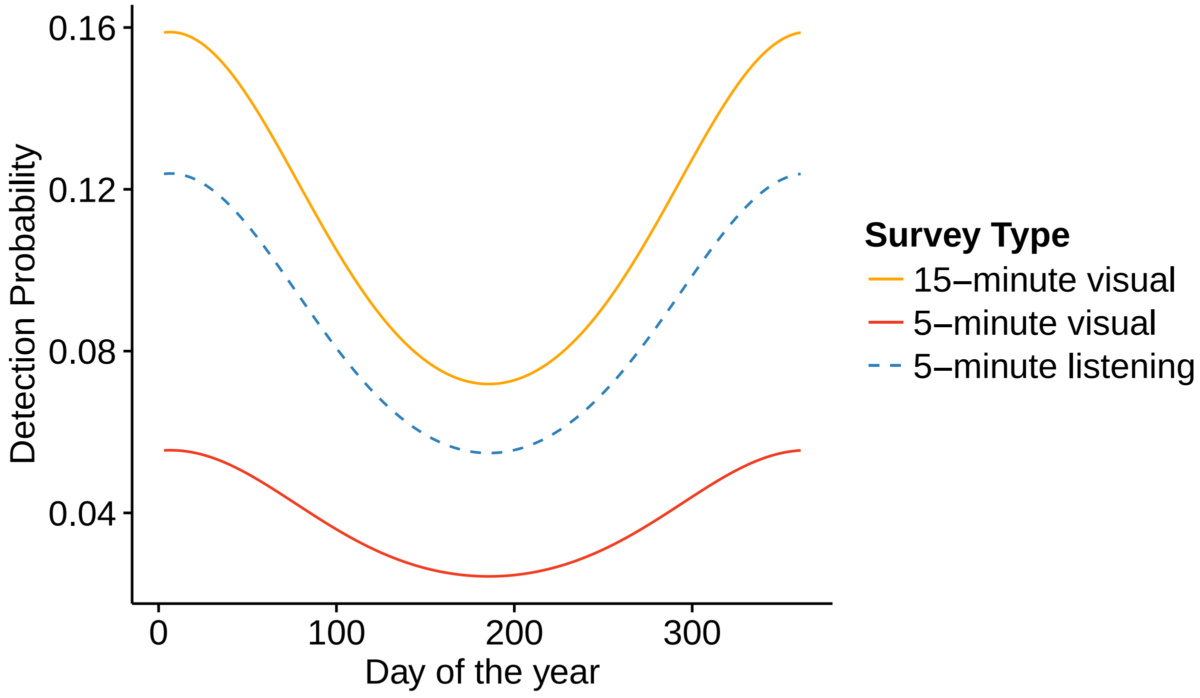


Figure 2. Seasonal effects on detection probability in listening and visual surveys

Visual surveys

In visual surveys detection probability was again affected by season, with higher detection probability in summer (Fig. 2A). Visual detection probability was also positively associated with temperature, although the effect in this case was smaller than it was for listening surveys (Fig. 3A; Table 1). Again, time after sunset and wind negatively affected detection probability (Fig. 3B & D). Moon illumination positively affected detection in visual surveys, but this was a very small effect (Table 1). Increased observer experience had a strong positive effect on detection probability in visual surveys (Fig. 3C).

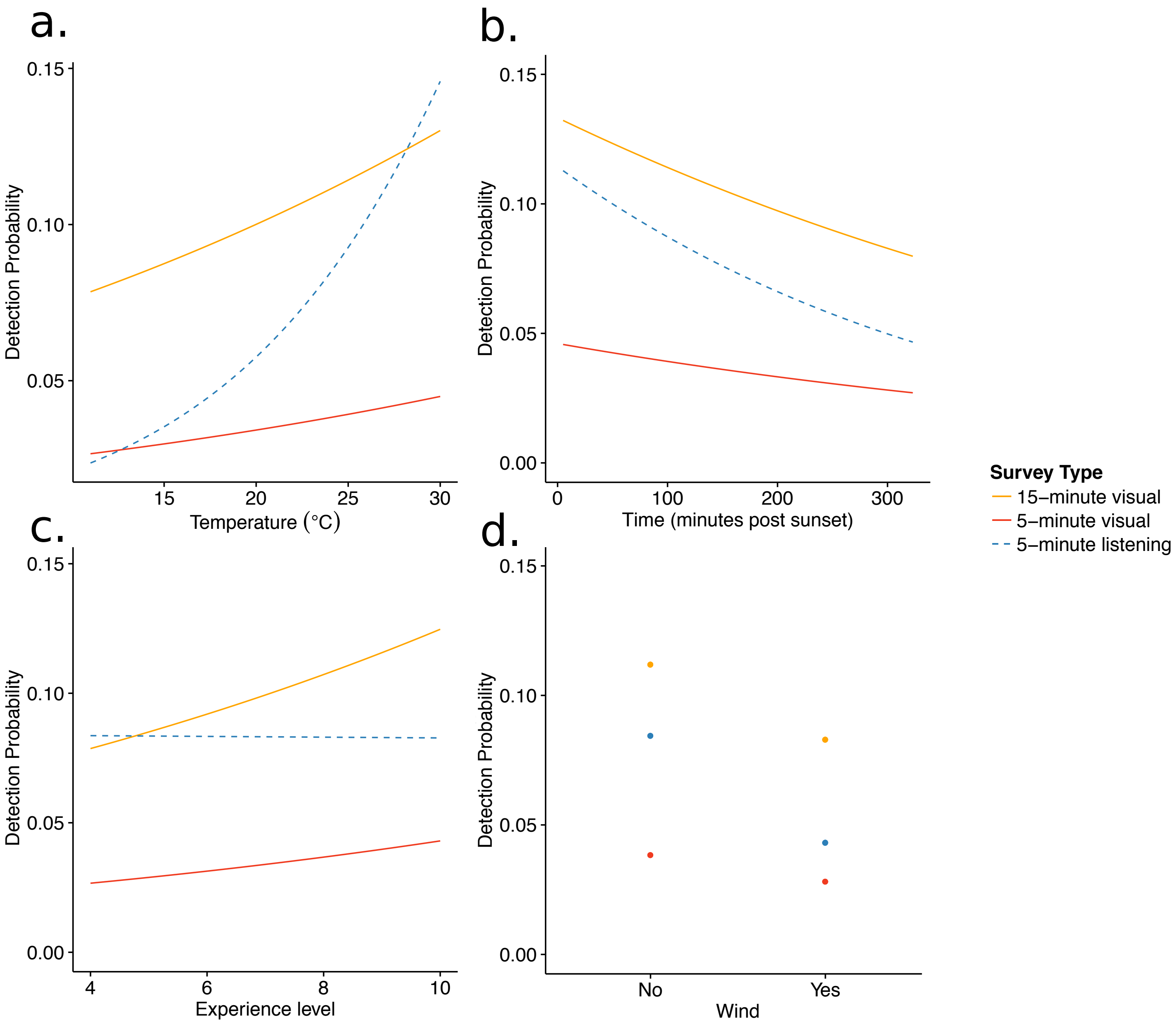


Figure 3. The predicted effects (presented as means of the Posterior Marginal Distribution) of **A.** temperature, **B.** time of night (minutes post sunset), **C.** observer experience level, and **D.** presence of wind on individual-level detection probability of *H. frenatus*.

Table 1. Factors affecting the detectability and estimated abundance of *H. frenatus* in (**A)** listening and (**B)** visual surveys. Estimates are derived from means of the Posterior Marginal Distribution. Asterisks indicate credible intervals that do not overlap zero. (Note: Wind is a two-level factor, while all other variables are continuous).

	Model	Parameter		Estimate	95% Credible Interval
a. **LISTENING**	Abundance	Distance from Urban Edge*		-0.0028	-0.0035 – -0.0022
		Colonisation time		0.0217	-0.0657 – 0.1167
		Distance.Time*		0.0001	0.0001 – 0.0001
	Detection	Time of year*	Sine(Day)*	-0.3967	-0.4839 – -0.3096
			Cosine(Day)*	0.4649	0.2926 – 0.6345
		Temperature*		0.1028	0.0647 – 0.1430
		Time post sunset*		-0.0030	-0.0040 – -0.0020
		Moon Illumination		< 0.0001	< -0.0001 – < 0.0001
		Obs. experience		-0.0019	-0.0384 – 0.0345
		Wind*		-0.7152	-0.8616 – -0.5701
b. **VISUAL**	Abundance	Distance from Urban Edge*		-0.0026	-0.0032 – -0.0020
		Colonisation time		0.0147	-0.0879 – 0.1220
		Distance.Time*		0.0001	0.00005 – 0.0001
	Detection	Time of year*	Sine(Day)	0.0281	-0.0428 – 0.0994
			Cosine(Day)*	0.4450	0.3145 – 0.5752
		Temperature*		0.0297	0.0032 – 0.0569
		Time post sunset*		-0.0018	-0.0026 – -0.0010
		Moon Illumination*		< 0.0001	< 0.0001 – 0.0001
		Obs. experience*		0.0854	0.0557 – 0.1156
		Wind*		-0.3316	-0.4477 – -0.2171

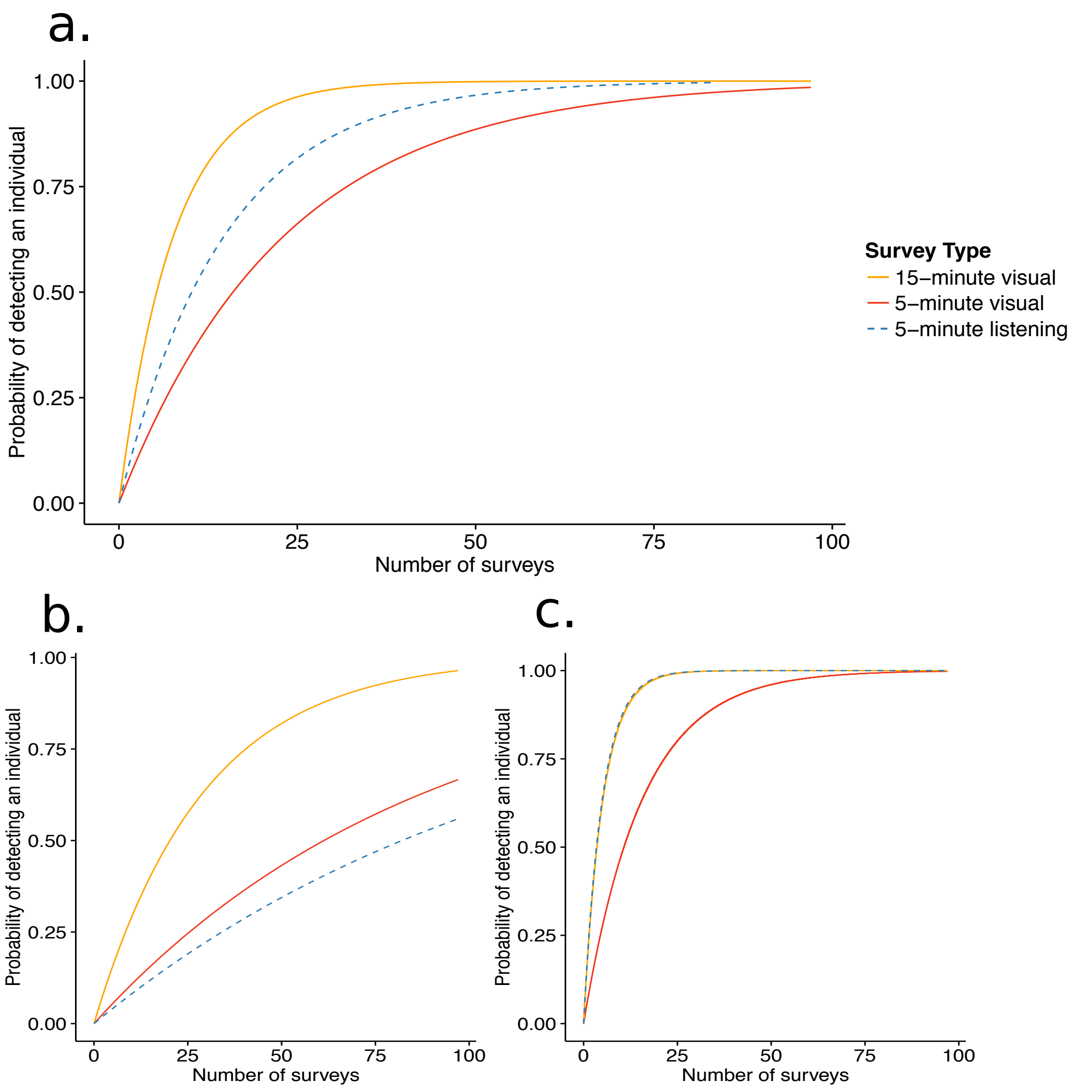


Figure 4. The probability of detecting an individual *H. frenatus* in 5-minute listening surveys, 5-minute visual surveys and 15-minute visual surveys against the number of surveys needed for detection in **A.** average survey conditions (March 16th, 111 minutes after sunset, no wind, 24°C), **B.** poor survey conditions (July 4th, 242 minutes after sunset, wind, 16°C) and **C.** good conditions (January 7th, 0 minutes after sunset, no wind, 32°C).

Survey effort

In Figure 4 we show the relationship between survey effort and individual-level detection of *H. frenatus* in a range of survey conditions. Overall, we found that choosing appropriate times to survey can result in a five-fold decrease in the number of surveys required for individual-level detection of *H. frenatus* (Fig. 4). In average conditions (Fig. 4A: March 16th, 111 minutes after sunset, no wind, 24°C), detection probability was highest for listening surveys, with five-minute listening surveys outperforming five-minute visual surveys. In poor survey conditions (Fig. 4B: July 4th, 242 minutes after sunset, wind, 16°C), detection probability was higher in visual surveys than listening surveys, while in optimal survey conditions (Fig. 4C: January 7th, 0 minutes after sunset, no wind, 32°C), five-minute listening surveys were substantially more efficient, being just as effective as 15-minute visual surveys.

Discussion

Most studies of detection assess population-level detection probability. For invasive species surveillance and monitoring, however, we are often interested in detecting single individuals before populations become firmly established. By assessing the factors that affect individual-level detection it is possible to increase the efficiency of surveys as well as allowing us to quantify our certainty that the organism is really absent.

Hemidactylus frenatus is a widespread invasive species, present in many tropical and subtropical areas around the world. To date there is little understanding of the factors that affect detection of this species, or whether visual or listening survey techniques are more efficient. Our data from 590 surveys across 50 sites and 12 months allowed us to assess which factors affect individual-level detection of *H. frenatus* in listening and visual surveys, while accounting for estimated abundance within each survey area. Our estimates of abundance were derived from the number of geckos recorded during 12 repeat visits to each site, as well as the abiotic conditions during each survey. While mark-recapture surveys are likely to give a better estimate of abundance, this method is costly and time consuming, and our main goal was to understand which factors affect detection within a defined survey area, not to estimate population size in a region.

We found that the ideal time to survey for *H. frenatus* in both listening and visual surveys was on warm nights in summer, not long after sunset. Choosing to survey in these conditions can increase detection probability at least three-fold over winter surveys late on cool nights (Fig. 4), and so result in substantially lower survey effort for a given detection probability. Our results agree with and extend previous work, which found that visual detection probability of *H. frenatus* decreases later in the night (Lardner et al. 2015). However, early research on calling behaviour found that *H. frenatus* call more later in the night (Marcellini 1974), which is the opposite to what we observe here. This earlier study, however, was conducted over only two months and it is possible that unmeasured factors (e.g., season) may have affected calling behaviour.

The positive effect of temperature was expected for this ectothermic species. Temperature, however, had a more pronounced effect on detection in listening surveys, than visual surveys (Fig. 2A), and at very high temperatures of approximately 28 – 30 °C, detection probability of *H. frenatus* was higher in five-minute listening surveys than in 15-minute visual surveys (Fig. 3A). Listening surveys are, therefore, likely to yield high detection probability in warmer parts of the introduced range of *H. frenatus*, while in cooler conditions visual surveys are likely to be more effective.

We also observed seasonal variation in detection probability, with detection being more likely during the summer months (Fig. 2A). This likely reflects the fact that breeding activity is more intense in summer. Although *H. frenatus* breed year round in the tropics (Church, 1962, Barnett et al. 2017), breeding peaks in the warmer parts of the year (Mckay and Phillips, 2012, Barnett et al. 2017). It is likely that breeding involves greater activity in *H. frenatus*, including increased calling; however, calling biology is poorly resolved.

The presence of wind negatively affected both visual and auditory detection probability of *H. frenatus.* Auditory detection, however, was more strongly affected by wind than visual detection (Fig. 3D), which may reflect not only reduced calling activity of geckos in windy conditions, but also the difficulty in hearing vocalisations over wind. Wind is widely cited as decreasing detection probability of reptiles and amphibians (Christy et al. 2010, Olson et al. 2012, Lardner et al. 2015).

The effect of moon illumination on detection probability is unlikely to be biologically important in this system. Moon illumination had no discernable effect on detection probability of *H. frenatus* in listening surveys, and for visual surveys the effect of moon illumination was very close to zero (Table 1). Nonetheless, previous research found moon phase significantly affected visual detection of *H. frenatus* on Guam, with fewer geckos seen on moonlit nights (Lardner et al. 2015). To date there has been little consensus as to how moon illumination affects the activity and detection probability of nocturnal herpetofauna (Christy et al. 2010, Weir et al. 2010, Sperry et al. 2013); and it is possible that the impact of moonlight is moderated by factors that vary between locations and species, such as predation risk, prey behaviour, or habitat structure.

Observer experience considerably increased the likelihood of visual detection for *H. frenatus*, but did not affect detection probability in listening surveys. The multiple chirp call of *H. frenatus* is loud and distinctive, and can be learned quickly by listening to recordings. Learning to recognise the eye-shine of small geckos, by contrast, can be a time-consuming process. Although Metson *et al.* (2012) found that false positive errors were common for inexperienced observers in frog auditory surveys, participants in their study were listening for the calls of multiple different species. In our study, assistants only needed to focus on learning one very distinctive call, which undoubtedly reduces error. Furthermore, although some native geckos in Australia (e.g. *Gehyra* spp.) also use vocalisations to communicate, these calls are very quiet and almost inaudible to the human ear (Phongkangsananan et al. 2014) , so there is no chance of confusing them with the loud and distinctive call of *H. frenatus*.

It is important to note that although we investigated the impacts of abiotic conditions on individual-level detection of *H. frenatus* (i.e., the probability of detecting one animal, irrespective of population size), the true probability of detecting an individual depends on biotic factors specific to that animal—including, age, sex, body condition and stage of the reproductive cycle (Conway et al. 2008, Christy et al. 2010)—and mark–recapture studies are necessary to assess how these factors affect detection.The current study, however, is focussed on identifying the most effective detection method across different abiotic conditions, and we do not address between individual differences in detection probability, although these are undoubtedly important.

Survey effort

We found that multiple surveys are necessary for reliable detection of *H. frenatus* in natural environments (Fig. 4). This is not surprising, and may explain why *H. frenatus* have gone unnoticed in natural habitats for so long. Nonetheless, understanding the efficiency of survey techniques in different conditions can increase the likelihood of detection. We found that choosing to survey in optimal conditions can result in a five-fold decrease in the number of surveys needed for accurate detection of *H. frenatus.* However, even in good survey conditions, to confirm an absence of this species (with a detection probability of 95%) given that no individuals are detected would require 15 x 15-minute visual surveys, or 15 x 15-minute listening surveys (Fig. 4). In Figure 4 we compare individual-level detection probability in listening and visual surveys, and show that five-minute listening surveys are more effective than five-minute visual surveys in average to good conditions, while in poor survey conditions visual surveys yield the highest likelihood of detection. Therefore, pairing listening and visual surveys gives a robust method for detecting small populations of *H. frenatus* across a range of conditions.

Previous work indicates that *H. frenatus* may persist at low abundance in natural habitats for many years before further range expansion and population growth (Barnett et al. 2017). Detecting these small populations is important for a number of reasons. First, over time these small populations of *H. frenatus* are likely to grow, spreading further into natural areas, with invasion driven by propagule pressure and time (Barnett et al. 2017). Second, at most of our study sites in the Townsville region *H. frenatus* has become abundant in natural habitats, now greatly outnumbering all native gecko species combined (Barnett et al. 2017), and *H. frenatus* has been demonstrated to cause declines and extinctions in native geckos elsewhere in its introduced range (Bolger and Case 1992, Petren and Case 1998, Cole et al. 2005, Hoskin 2011). Therefore, if small populations are detected early enough, it may be possible to inhibit range expansion and impacts in natural habitats. Detecting small populations of *H. frenatus* may be particularly relevant in areas of conservation concern, such as islands and habitat for threatened or range-restricted geckos. To these ends, our work here offers critical insights into how to design effective surveillance surveys.

Implications

We found that multiple site visits are necessary for reliable detection of *H. frenatus* in natural environments. Listening surveys could be a useful and cost effective method of surveillance, especially for unskilled observers in warmer parts of the introduced range. Listening surveys, however, do not provide the same important ecological information as visual surveys, which give insights into population structure, reproduction and the presence of co-occurring species. Furthermore, listening surveys are more strongly affected by temperature, time of night and wind, and in poor survey conditions detection probability is higher in visual surveys. Overall, our 20-minute survey, consisting of a five-minute listening survey and a 15-minute visual search is well designed to maximise detection probability of *H. frenatus* across a range of abiotic conditions and survey abilities.

Our results will assist ecologists and members of the public to conduct more effective surveys for *H. frenatus* in both natural and urban habitats, and facilitate more accurate distribution mapping of this highly invasive species. Furthermore, a greater understanding of detection probability could assist managers in monitoring areas of conservation concern. Effective removal of *H. frenatus* may also be possible in these regions if small populations are identified early enough, because colonisation success in natural environments appears strongly controlled by propagule pressure in *H. frenatus* (Barnett et al. 2017).

By investigating the relationship between environmental conditions and individual-level detection probability we have identified an effective survey technique for small populations of an invasive gecko. We have also shown that binomial mixture models are highly effective for assessing which factors affect individual-level detection probability, as well as estimating abundance within the areas surveyed.

References

Barnett, L. K. et al. 2017. Going feral: Time and propagule pressure determine range expansion of Asian house geckos into natural environments. - Austral Ecol. 42: 165–175.

Bolger, D. T. and Case, T. J. 1992. Intra- and interspecific interference behaviour among sexual and asexual geckos. - Anim. Behav. 44: 21–30.

Boulinier, T. et al. 1998. Estimating species richness: the importance of heterogeneity in species detectability. - Ecology 79: 1018–1028.

Britton, J. R. et al. 2011. Quantifying imperfect detection in an invasive pest fish and implications for conservation management. - Biol. Conserv. 1: 1–24.

Bruemmer, C. M. et al. 2010. Epidemiology of squirrelpox virus in grey squirrels in the UK. - Epidemiol. Infect. 138: 941–950.

Case, T. J. et al. 1994. Invasions and competitive displacement among house geckos in the tropical Pacific. - Ecology 75: 464–477.

Chapple, D. G. et al. 2016. Biosecurity of exotic reptiles and amphibians in New Zealand building upon Tony Whitaker’s legacy. - J. R. Soc. New Zeal. 46: 1–9.

Christy, M. T. et al. 2010. Modelling detection probabilities to evaluate management and control tools for an invasive species. - J. Appl. Ecol. 47: 106–113.

Church, G. 1962. The reproductive cycles of the Javanese house geckos, Cosymbotus platyurus, *Hemidactylus frenatus*, and *Peropus mutilatus*. - Am. Soc. Ichthyol. Herpetol. 1962: 262–269.

Cole, N. C. et al. 2005. The need for enemy-free space: the impact of an invasive gecko on island endemics. - Biol. Conserv. 125: 467–474.

Conway, C. J. et al. 2008. Factors affecting detection of Burrowing Owl nests during standardized surveys. - J. Wildl. Manage. 72: 688–696.

Dejean, T. et al. 2012. Improved detection of an alien invasive species through environmental DNA barcoding: the example of the American Bullfrog Lithobates catesbeianus. - J. Appl. Ecol. 49: 953–959.

Gormley, A. M. et al. 2011. Using presence-only and presence-absence data to estimate the current and potential distributions of established invasive species. - J. Appl. Ecol. 48: 25–34.

Green, S. J. et al. 2013. Habitat complexity and fish size affect the detection of Indo-Pacific lionfish on invaded coral reefs. - Coral Reefs 32: 413–421.

Hoskin, C. J. 2011. The invasion and potential impact of the Asian House Gecko (*Hemidactylus frenatus*) in Australia. - Austral Ecol. 36: 240–251.

Keith, J. M. and Spring, D. 2013. Agent-based Bayesian approach to monitoring the progress of invasive species eradication programs. - Proc. Natl. Acad. Sci. U. S. A. 110: 13428–13433.

Lardner, B. et al. 2015. Detection rates of geckos in visual surveys: turning confounding variables into useful knowledge. - J. Herpetol. 0: 522–532.

Lei, J. and Booth, D. T. 2014. Temperature, field activity and post-feeding metabolic response in the Asian house gecko, *Hemidactylus frenatus*. - J. Therm. Biol. 45: 175–80.

Lever, C. 2003. Naturalized reptiles and amphibians of the world. - Oxford University Press.

Mackenzie, D. I. 2005. Was it there? Dealing with imperfect detection for species presence/absence data. - Aust. New Zeal. J. Stat. 47: 65–74.

MacKenzie, D. I. et al. 2002. Estimating site occupancy rates when detection probabilities are less than one. - Ecology 83: 2248–2255.

Marcellini, D. L. 1974. Acoustic behavior of the Gekkonid lizard, *Hemidactylus frenatus*. - Herpetologica 30: 44–52.

Marcellini, D. L. 1977. The function of a vocal display of the lizard Hemidactylus frenatus (Sauria: Gekkonidae). - Anim. Behav. 25: 414–417.

Mckay, J. L. and Phillips, B. L. 2012. Climatic determinants of the reproductive timing in the Asian house gecko, *Hemidactylus frenatus* Dumeril and Bibron (Gekkonidae). - Raffles Bull. Zool. 60: 583–588.

Metson, G. S. et al. 2012. Experimental investigation of false positive errors in auditory species occurrence surveys. - Ecol. Appl. 22: 1665–1674.

Olson, C. A. et al. 2012. Detection probabilities of two introduced frogs in Hawaii: Implications for assessing non-native species distributions. - Biol. Invasions 14: 889–900.

Oppel, S. et al. 2014. Improving aquatic warbler population assessments by accounting for imperfect detection. - PLoS One 9: 1–7.

Pellet, J. and Schmidt, B. 2005. Monitoring distributions using call surveys: estimating site occupancy, detection probabilities and inferring absence. - Biol. Conserv. 123: 27–35.

Petren, K. and Case, T. J. 1998. Habitat structure determines competition intensity and invasion success in gecko lizards. - Proc. Natl. Acad. Sci. U. S. A. 95: 11739–11744.

Phongkangsananan, N. et al. 2014. Chatty females and quiet males: complex vocal communication in the Northern Dtella, *Gehyra dubia*. - Herpetol. Conserv. Biol. 9: 285–296.

Plummer, M. 2016. rjags: Bayesian graphical models using MCMC. in press.

R Core Team 2016. R: A language and environment for statistical computing.: R

Foundation for Statistical Computing, Vienna, Au.

Rödder, D. et al. 2008. Predicting the potential distribution of two alien invasive Housegeckos (Gekkonidae: *Hemidactylus frenatus*, *Hemidactylus maboui*a). - North. West. J. Zool. 4: 236–246.

Royle, J. A. 2004. N-mixture models for estimating population size from spatially replicated counts. - Biometrics 60: 108–115.

Royle, A. J. and Nichols, J. D. 2003. Estimating abundance from repeated presence–absence data or point counts. - Ecology 84: 777–790.

Somaweera, R. et al. 2010. Frogs under friendly fire: How accurately can the general public recognize invasive species? - Biol. Conserv. 143: 1477–1484.

Sperry, J. H. et al. 2013. Effects of temperature, moon phase, and prey on nocturnal activity in ratsnakes: an automated telemetry study. - J. Herpetol. 47: 105–111.

Stolwijk, A. M. et al. 1999. Studying seasonality by using sine and cosine functions in regression analysis. - J. Epidemiol. Community Health 53: 235–238.

Tang, Y.-Z. et al. 2001. Advertisement calls and their relation to reproductive cycles in *Gekko gecko* (Reptilia, Lacertilia). - Copeia 2001: 248–253.

Tingley, R. et al. 2014. European newts establish in Australia, marking the arrival of a new amphibian order. - Biol. Invasions in press.

Toft, N. et al. 2007. Assessing the convergence of Markov Chain Monte Carlo methods: An example from evaluation of diagnostic tests in absence of a gold standard. - Prev. Vet. Med. 79: 244–256.

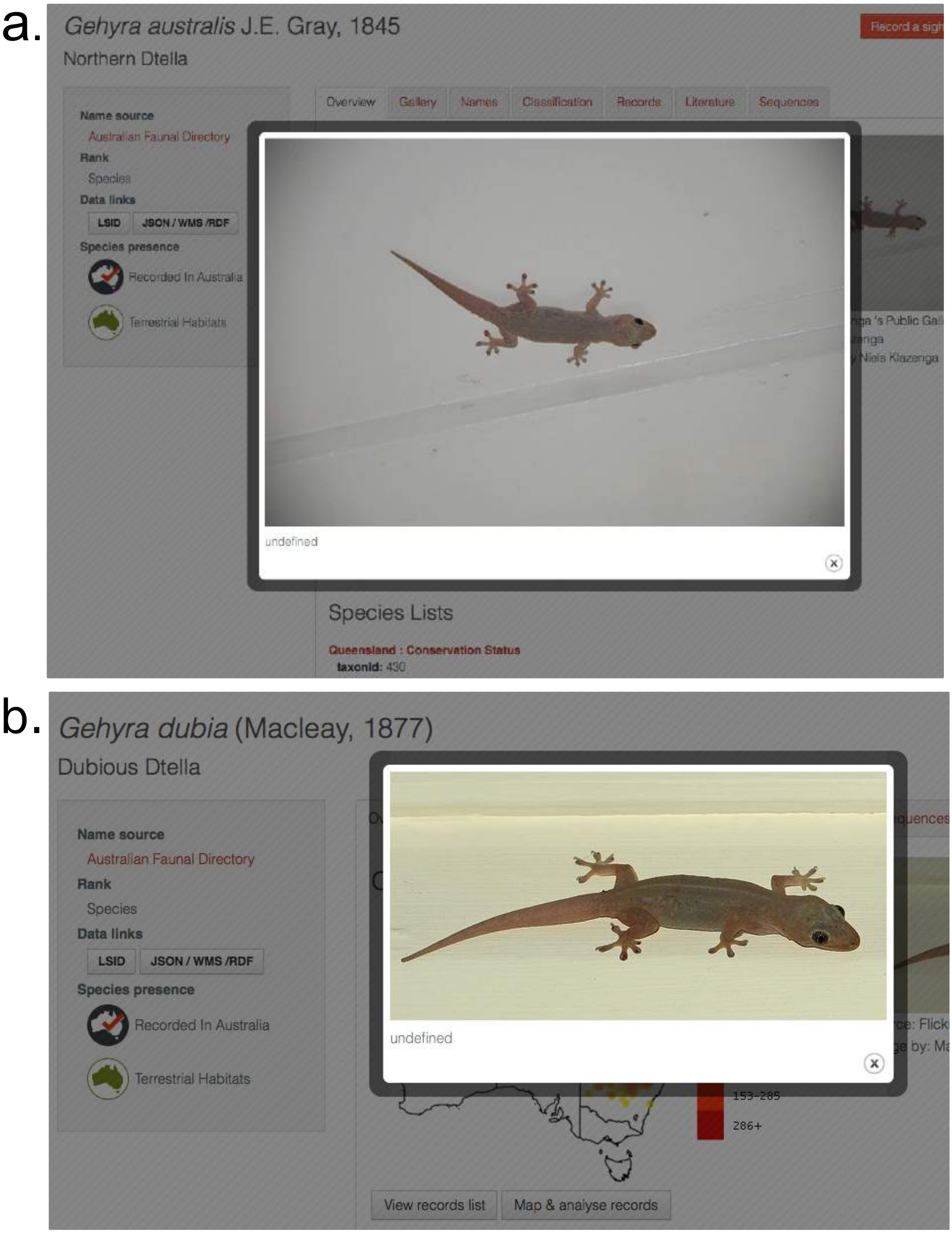
Weir, L. A. et al. 2010. Modeling anuran detection and site occupancy on North American Amphibian Monitoring Program (NAAMP) routes in Maryland. - J. Herpetol. 39: 627–639.

Wenger, S. J. and Freeman, M. C. 2008. Estimating species occurrence, abundance, and detection probability using zero-inflated distributions. - Ecology 89: 2953–9.

Yamamoto, Y. and Ota, H. I. 2006. Long-term functional sperm storage by a female common house gecko, *Hemidactylus frenatus* , from the Ryukyu Archipelago, Japan. - Curr. Herpetol. 25: 39–40.

Zozaya, S. M. et al. 2015. Invasive house geckos are more willing to use artificial lights than are native geckos. - Austral Ecol. 40: 982–987.

Chapter 5
Supplementary Material



Gehyra australis J.E. Gray, 1845
Northern Dtella

Name source
Australian Faunal Directory
Rank
Species
Data links
Species presence
Recorded In Australia
Terrestrial Habitats

Species Lists
Queensland : Conservation Status
taxonId: 430

Gehyra dubia (Macleay, 1877)
Dubious Dtella

Name source
Australian Faunal Directory
Rank
Species
Data links
Species presence
Recorded In Australia
Terrestrial Habitats

Supplementary Figure 1. Due to visual similarities *H. frenatus* are often mistaken for native *Gehyra* geckos in Australia. This figure shows screen grabs from the excellent 'Atlas of Living Australia' website. Two native *Gehyra* species profiles had *H. frenatus* photos. These errors have been corrected on ALA.

Supplementary Table 1. Minimally-informative priors used in binomial mixture models of *H. frenatus* detection

Model	Parameter	Prior Distribution
Abundance	Distance from Urban Edge	dnorm(0,1.0e-6)
	Colonisation time	dnorm(0,1.0e-6)
	Distance.Time	dnorm(0,1.0e-6)
	muN	dnorm(0,1.0e-6)
	tauN	dgamma(0.001, 0.001)
Detection	Cosine(day	dnorm(0,1.0e-6)
	Sine(day)	dnorm(0,1.0e-6)
	Temperature	dnorm(0,1.0e-6)
	Time post sunset	dnorm(0,1.0e-6)
	Moon Illumination	dnorm(0,1.0e-6)
	Obs. experience	dnorm(0,1.0e-6)
	Wind	dnorm(0,1.0e-6)

Chapter 6

Thesis Summary

Hemidactylus frenatus is a highly successful invasive species, with an extensive global distribution in the tropics and subtropics (Lever, 2003; Hoskin, 2011). Close associations to humans, however, have led to the belief that, with the exception of islands, *H. frenatus* is restricted to highly-modified anthropogenic habitats in its introduced range (Vanderduys and Kutt 2013).

I investigated the potential for *H. frenatus* to establish in natural habitats in the Townsville region of northern Australia, and assessed whether range expansion of this species into woodland environments is associated with demographic factors, environmental factors, morphological changes and/or parasite exposure. I also addressed the potential impact of *H. frenatus* on native geckos in these environments by investigating patterns of co-occurrence and parasite transmission between native and invasive geckos. Lastly, I evaluated the efficiency of listening and visual surveys in detecting *H. frenatus*, and assessed the best conditions for *H. frenatus* detection in natural habitats. Results of this research will be important for the management of *H. frenatus* in Australia and elsewhere in the invasive range, and will provide insight into the factors that control range expansion of human-associated species into natural environments.

Here, I provide the first evidence for large, self-sustaining populations of *H. frenatus* in natural environments on the Australian mainland. I recorded *H. frenatus* at 2 km from the urban edge at nine out of ten of the invasion fronts and *H. frenatus* were more abundant than native geckos at most woodland sites. Populations of *H. frenatus* in natural habitats appeared to be well established with similar body conditions, sex ratios and proportions of reproductive females to urban populations. This is the first study to quantify establishment of *H. frenatus* populations in natural habitats outside of islands and these results indicate that, at least in some areas, *H. frenatus* may be a greater threat to native geckos in natural ecosystems than is currently acknowledged.

Range Expansion

Range expansion of invasive species is unpredictable and is affected by propagule pressure, adaptations, parasite exposure, habitat boundaries, competition with native species and many other factors (Rissler et al. 2000, Burton et al. 2010, Phillips et al. 2010). I found that fine-scale spread of *H. frenatus* into natural habitats was affected by the same factors that control establishment success of invasive species on larger scales – propagule pressure and time (Lockwood et al. 2005, Simberloff 2009). The number of buildings in the adjacent urban area did not affect establishment in woodland habitats, indicating that even a few isolated structures could act as a stepping-stone for *H. frenatus* to colonise natural areas (Barnett et al., 2016).

I found that although *H. frenatus* were highly abundant in natural environments, they were more abundant in adjacent urban areas. These differences in relative abundance have likely arisen because urban habitats have a higher carrying capacity for arboreal geckos—i.e., more retreat sites and higher insect abundance—rather than a detection bias between habitats. While detectability of *H. frenatus* may be greater in urban areas, we tried to minimise this bias by not counting geckos found on houses or walls (where tens of geckos are often found congregating around lights). We did, however, count geckos on poles, signs and other human objects that resembled trees, and on natural objects in urban environments. Furthermore, as urban areas are more structurally complex, it is likely that for every gecko seen, many more are hiding in places that are impossible to see during surveys, e.g. in roofs, inside buildings and under ornamental plants. Therefore, there is almost certainly a higher carrying capacity for *H. frenatus* in urban environments than the surrounding woodland. Lower abundance in woodland environments might lead to the belief that *H. frenatus* are not a threat in these habitats, but it is important to note that *H. frenatus* persist in higher abundances than native geckos in natural environments, and it is vital to investigate the factors that control range expansion of this species into natural habitats.

The coarse structure of the natural habitats studied (which ranged from high-quality to degraded woodland) did not affect range expansion of *H. frenatus,* showing that habitat degradation is not essential for this species to thrive in natural environments. Nonetheless, this study was restricted to open woodland habitats within a relatively small geographic area, and it is unclear whether *H. frenatus* can thrive in other habitat types, such as rainforest, wet sclerophyll forests or rock-dominated habitats, in Australia. Furthermore, while our measure of tree density gave an indication of habitat degradation—with visually more degraded woodland having a lower tree density score—a more comprehensive study of plant species at each site could give a better indication of which habitat features promote *H. frenatus* invasion. Future work should address *H. frenatus* habitat preferences within natural environments in greater detail and over a broader geographic range.

My results indicate that—irrespective of coarse habitat structure or size of the urban area—with sufficient immigration from urban populations, *H. frenatus* can eventually become self-sustaining in woodland habitats and continue to spread into natural environments. As such, the longer that *H. frenatus* have been present in an urban area, the more likely that large populations will be found farther from the urban edge (Barnett et al., 2016). Although my study was limited to sites within 2 km of the urban edge, these populations were firmly established, indicating that given enough time *H. frenatus* are likely to spread much farther into natural environments.

Figure 1 shows the interactive effect of colonisation time and distance from the urban edge on the predicted abundance of *H. frenatus* in woodland habitats. At the minimum colonisation time of 16 years there is a negative effect of distance on *H. frenatus* abundance; however, at transects where *H. frenatus* have been present for 27 years or more, this effect is reversed (Fig. 1). Furthermore, it is important to note that the oldest population studied here established in approximately 1980, while in the Northern Territory *H. frenatus* have been present since the 1960s. In such places *H. frenatus* are likely to have spread much farther into natural environments, and future research should focus on expansion and potential impacts in these areas.

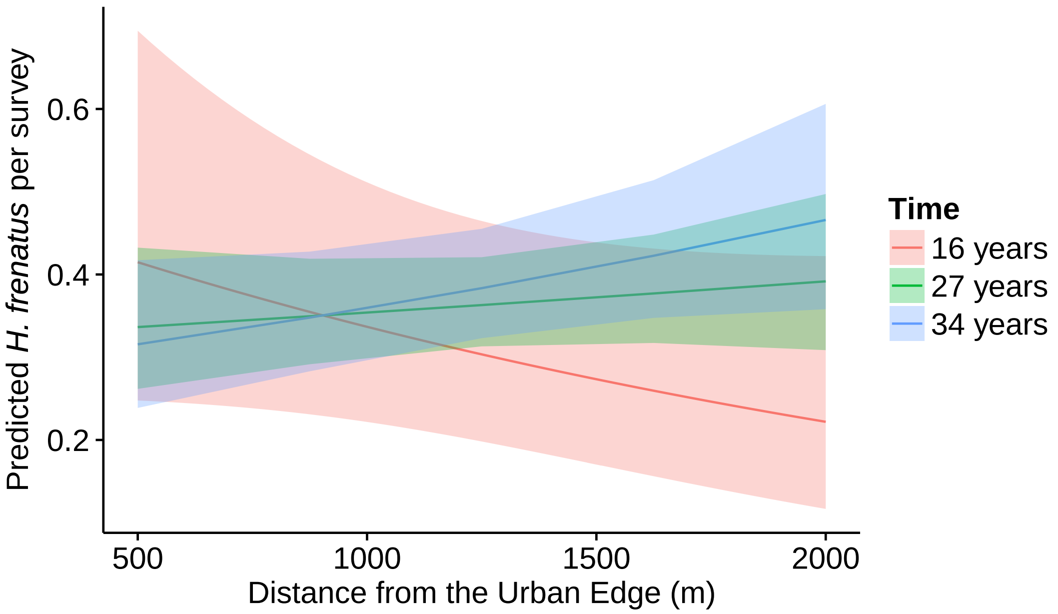


Figure 1. The predicted effect of the interaction between colonisation time (the estimated date of *H. frenatus* establishment in the urban area) and distance from the urban edge on the abundance of *H. frenatus* in woodland environments from 500 m to 2000 m from the urban edge.

Time and propagule pressure (i.e. immigration from urban areas) may also facilitate morphological, physiological or behavioural adaptations to novel habitats, by increasing genetic diversity in invasive populations (Kolbe et al. 2004, Mukherjee et al. 2011). Morphological changes may be particularly important for species transitioning abrupt habitat boundaries like the urban–natural interface (Winchell et al. 2016). I documented divergence in body and toe morphology between urban and woodland populations of *H. frenatus* on multiple semi-independent invasion fronts. Some traits (e.g., male body size) differed consistently between urban and woodland habitats. However, for other traits (e.g., male body shape) the direction of trait shifts varied between woodland populations at different invasion fronts. This may be due to the fact that most urban environments share similar habitat features, whereas different natural habitats may be more variable in terms of thermal regime, habitat structure and biotic communities (Grimm et al. 2008, Lei and Booth 2014). So, while convergent trait shifts may be associated with colonisation of different urban environments (e.g.,Winchell et al., 2016), for invasive species establishing in different natural habitats divergent trait shifts may be likely (e.g., Losos et al., 1997).

While I found evidence of phenotypic divergence in woodland populations of *H. frenatus,* it is unclear whether the phenotypic changes observed here have arisen through plasticity or adaptive evolution. It is also unclear what the adaptive significance of these changes might be, and why some traits vary consistently between urban and woodland populations, while others were highly dependent on the invasion front studied. Future research should investigate whether phenotypic divergence in this system is the result of adaptive evolution or phenotypic plasticity, and assess the adaptive significance of morphological changes through laboratory studies of functional morphology (e.g., by testing gripping ability on different surfaces).

Parasite exposure can also affect range expansion of invasive species. Parasite release on the invasion front may facilitate range expansion (Phillips et al. 2010), while it is possible that exposure to novel parasites in a new habitat could inhibit spread (Case and Taper 2000). During range expansion into natural environments, *H. frenatus* on the invasion front experience release from co-evolved *Geckobia* mites, but are exposed to nymphs of a native pentastome parasite, *Waddycephalus* sp., which is absent in inner urban areas, but present in woodland habitats and at the urban edge (Coates et al., in press).

While the prevalence of *Geckobia* mites decreased with distance from the urban edge, I found no evidence that mites affect the body condition or lifespan of *H. frenatus*, and release from mites on the invasion front is, therefore, unlikely to affect range expansion. While mites can cause skin irritation in other lizards (Goldberg and Bursey 1991, Goldberg and Holshuh 1992), there is currently no evidence of mites affecting condition in wild gecko populations (Hanley et al. 1995).

Native *Waddycephalus* nymphs also had no effect on body condition; however, it is possible that *Waddycephalus* affect the lifespan of *H. frenatus*, as I found fewer large individuals with high-intensity *Waddycephalus* infections. Although data on *Waddycephalus* are lacking, it is clear that nymphs of other pentastomids can affect the health of the intermediate host in the process of burrowing from the stomach to the body wall (Paré 2008). However, it is also possible that some nymphs are lost as geckos age, either because they excyst, or because adult geckos have a way of shedding them. Future research should assess the impact of *Waddycephalus* nymphs on *H. frenatus* health, focussing on whether these parasites affect geckos in a way that is not expressed by a change in body condition.

Potential Impact

As well as affecting range expansion, parasites associated with invasive species can have severe impacts on native species in the introduced range (Andreou et al. 2012). In many of the natural environments studied, *H. frenatus* co-occur with and greatly outnumber native geckos (all species combined), highlighting the potential for parasite transmission and competition between invasive and native geckos. There was, however, no evidence of mite transmission between native and invasive geckos, with *H. frenatus* hosting introduced mites (*G. bataviensis* and *G. keegani*) and native geckos hosting native mite species. This was unexpected and may reflect host specificity in *Geckobia* mites, or limited interactions between *H. frenatus* and native geckos in these habitats. Mites introduced with *H. frenatus* (i.e., *Geckobia bataviensis* and *Geckobia keegani*) have been recorded infecting other gecko species (Heath and Whitaker 2015), and laboratory studies would be a useful way of addressing host specificity and host competency for mites in this system.

The fact that *H. frenatus* can host native *Waddycephalus* nymphs is concerning for native hosts of this parasite (i.e., native geckos and snakes). *Hemidactylus frenatus* appear to be providing the same trophic link as native geckos in this system and may, therefore, increase the prevalence of *Waddycephalus* nymphs in native gecko and snake hosts through parasite spillback. Future work should investigate whether this is the case by assessing *Waddycephalus* prevalence in native hosts in areas where they co-occur with *H. frenatus* compared to areas where *H. frenatus* are absent. Furthermore, while I observed *Waddycephalus* nymphs infecting native geckos (*Gehyra dubia*), to date the prevalence and impact of *Waddycephalus* in native Australian geckos remains unknown and this provides an intriguing topic for future research (Barton 2007).

Although I did not directly assess competition between *H. frenatus* and native geckos, co-occurrence data provide a strong argument that this should be a priority for future research. At most transects, *H. frenatus* were more abundant than native geckos in natural environments. However, even at the one transect where *H. frenatus* have not yet spread beyond the urban edge (Mount Elliott), native geckos are less abundant than are *H. frenatus* at woodland sites elsewhere. This indicates that *H. frenatus* can reach unusually high abundances in woodland habitats and could, therefore, affect native species through resource competition and parasite spillback. This is very concerning, considering Australia is a global centre for gecko diversity and endemism, with many threatened and range restricted species (Hoskin 2011, Macdonald 2015).

Detection

To accurately understand the impact of any invasive species, it is vital to appreciate the extent of its distribution. Detection of invasive species is imperfect and this presents a major challenge in detection, quarantine and control activities (Keith and Spring 2013). I used binomial mixture models to assess the factors that affect detection of *H. frenatus* in natural environments in both listening and visual surveys, and found that detection of *H. frenatus* is strongly controlled by abiotic factors, most notably, season, temperature, wind, and time of day.

The best time to survey for *H. frenatus* was on warm, still nights, not long after sunset. I found that multiple site visits are necessary to detect small populations of *H. frenatus* in natural environments, especially in sub-optimal survey conditions. Listening surveys are a useful and efficient detection method, especially for inexperienced observers. In poor survey conditions, however, detection probability was greater in visual surveys. Overall, pairing five-minute listening and 15-minute visual surveys should increase the likelihood of detecting small populations of *H. frenatus* in different survey conditions and among surveyors of different experience.

Conclusion

Overall, data from this study will assist in understanding the potential impact of *H. frenatus* in Australia and will guide efforts to control the spread of this species in natural environments. I found large, self-sustaining populations in woodland habitats. In many of these habitats, *H. frenatus* were more abundant than native geckos and hosted native *Waddycephalus* parasites, indicating that parasite spillback to native hosts is a potential risk in this system. These results suggest that more concern should be shown when *H. frenatus* is introduced to new areas.

Once *H. frenatus* are established in natural environments it is unlikely that control efforts (e.g., physically removing individual geckos) will be effective. My results, however, indicate that limiting the spread of *H. frenatus* into certain areas could be possible through carefully planned management actions. For example, if *H. frenatus* are introduced to an anthropogenic area that adjoins important habitat for a threatened gecko species, targeted removal from structures could prevent subsequent expansion into natural habitats because range expansion into natural environments is determined by urban population size and time. These efforts should be concentrated on areas of conservation concern, such as islands and other key habitat for threatened or range restricted geckos.

More broadly, this work has demonstrated that human-associated invasive species provide a useful system to assess range expansion over multiple semi-independent invasion fronts. The importance of propagule pressure and time in facilitating expansion of *H. frenatus* indicates that lag periods could be likely for other human associated species spreading into natural environments. Nonetheless, lag periods are a complex part of invasion and the length of lags may vary considerably between species and locations. The inherently lower abundances of human associated species in natural environments compared to urban areas can lead to them initially being overlooked as potential threats in natural habitats, which can result in perceived lags in range expansion. Furthermore, this study has shown that human associated species are difficult to detect in natural habitats and multiple site visits are necessary to detect small populations soon after establishment. Therefore, while propagule pressure and time were determinants of *H. frenatus* range expansion, future studies should address the factors that facilitate expansion of invasive species after lag periods in more detail, while accounting for detection probability and making comparisons across multiple species and locations.

References

Andreou, D. et al. 2012. Introduced pathogens and native freshwater biodiversity: a case study of Sphaerothecum destruens. - PLoS One 7: e36998.

Barnett, L. K. et al. 2017. Going feral: Time and propagule pressure determine range expansion of Asian house geckos into natural environments. - Austral Ecol. 42: 165–175.

Barton, D. P. 2007. Pentastomid parasites of the introduced Asian house gecko, *Hemidactylus frenatus* (Gekkonidae), in Australia. - Comp. Parasitol. 74: 254–259.

Burton, O. J. et al. 2010. Trade-offs and the evolution of life-histories during range expansion. - Ecol. Lett. 13: 1210–1220.

Case, T. J. and Taper, M. L. 2000. Competition, environmental gradients, gene flow and the coevolution of species' borders. - Am. Nat. 155: 583–605.

Coates, A. et al. 2017. Living on the Edge: Parasite Prevalence Changes Dramatically across a Range Edge in an Invasive Gecko. - Am. Nat. in press.

Goldberg, S. R. and Bursey, C. R. 1991. Integumental lesions caused by ectoparasites in a wild population of the side-blotched lizard (*Uta stansburiana*). - J. Wildl. Dis. 27: 68–73.

Goldberg, S. R. and Holshuh, H. J. 1992. Ectoparasite-induced lesions in mite pockets of the Yarrow's spiny lizard, *Sceloporus jarrovii* (Phrynosomatidae). - J. Wildl. Dis. 28: 537–541.

Grimm, N. B. et al. 2008. Global change and the ecology of cities. - Science (80-.). 319: 756–760.

Hanley, K. A. et al. 1995. Lower mite infestations in an asexual gecko compared with its sexual ancestors. - Evolution (N. Y). 49: 418–426.

Heath, A. C. G. and Whitaker, A. H. 2015. Mites (Acari: Pterygosomatidae, Macronyssidae) taken from lizards intercepted at the New Zealand border. - Syst. Appl. Acarol. 20: 739.

Hoskin, C. J. 2011. The invasion and potential impact of the Asian House Gecko (*Hemidactylus frenatus*) in Australia. - Austral Ecol. 36: 240–251.

Keith, J. M. and Spring, D. 2013. Agent-based Bayesian approach to monitoring the progress of invasive species eradication programs. - Proc. Natl. Acad. Sci. U. S. A. 110: 13428–13433.

Kolbe, J. J. et al. 2004. Genetic variation increases during biological invasion by a Cuban lizard. - Nature 431: 177–181.

Lei, J. and Booth, D. T. 2014. Temperature, field activity and post-feeding metabolic response in the Asian house gecko, Hemidactylus frenatus. - J. Therm. Biol. 45: 175–80.

Lever, C. 2003. Naturalized reptiles and amphibians of the world. - Oxford University Press.

Lockwood, J. L. et al. 2005. The role of propagule pressure in explaining species invasions. - Trends Ecol. Evol. 20: 223–8.

Losos, J. B. et al. 1997. Adaptive differentiation following experimental island colonization in Anolis lizards. - Nature 387: 70–73.

Macdonald, S. 2015. Australian Reptiles Online Database.

Mukherjee, A. et al. 2011. Brazilian peppertree (*Schinus terebinthifolius*) in Florida and South America: evidence of a possible niche shift driven by hybridization. - Biol. Invasions 14: 1415–1430.

Paré, J. a. 2008. An overview of pentastomiasis in reptiles and other vertebrates. - J. Exot. Pet Med. 17: 285–294.

Phillips, B. L. et al. 2010. Parasites and pathogens lag behind their host during periods of host range advance. - Ecology 91: 872–81.

Rissler, L. J. et al. 2000. Spatial and behavioral interactions between a native and introduced salamander species. - Behav. Ecol. Sociobiol. 48: 61–68.

Simberloff, D. 2009. The role of propagule pressure in biological invasions. - Annu. Rev. Ecol. Evol. Syst. 40: 81–102.

Vanderduys, E. P. and Kutt, A. S. 2013. Is the Asian house gecko, *Hemidactylus frenatus*, really a threat to Australia’s biodiversity? - Aust. J. Zool. 60: 361–367.

Winchell, K. M. et al. 2016. Phenotypic shifts in urban areas in the tropical lizard Anolis cristatellus. - Evolution (N. Y). 70: 1009–1022.