
UNDERSTANDING WHAT MAKES A LIZARD INVASIVE: THE ROLE OF BEHAVIOUR AND COGNITION

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*To my family,
my very beginning.*

*À minha família,
o meu iniciozinho.*

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STATEMENT OF ORIGINALITY

I certify that the work in this thesis entitled “Understanding what makes a lizard invasive: The role of behaviour and cognition” has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree to any other university or institution other than Macquarie University. I also certify that the thesis is an original piece of research and it has been written by me. Any help and assistance that I have received in my research work and the preparation of the thesis itself have been appropriately acknowledged.

In addition, I certify that all information sources and literature used are indicated in the thesis.

The research presented in this thesis was approved by the Macquarie University Animal Research Ethics Committee, reference numbers ARA2015/038 and ARA2017/004.

Permission to carry out field and lab work in Portugal was issued by the ICNF (“Instituto da Conservação da Natureza e das Florestas”, translated to Institute for Conservation of Nature and Forestry), reference numbers: 191/2016/CAPT, 695/2016/CAPT, 157/2017/CAPT, and 428/2017/CAPT.

Maria Isabel Vidigal Bettencourt Damas Moreira

Date 23.07.2018

LIST OF ORIGINAL PUBLICATIONS

The following original chapters have been written as stand-alone papers. One of these chapters is published and two of them are currently in review.

Chapter I

Isabel Damas-Moreira, Ana Pereira, Julia L Riley, D James Harris, and Martin J Whiting. **Is invasive potential linked to cognitive ability in a lizard?** *In preparation for submission.*

Chapter II

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Chapter III

Isabel Damas-Moreira, Julia L Riley, D James Harris, and Martin J Whiting. **Can behaviour explain invasion success? A comparison between sympatric invasive and native lizards.** *Manuscript in review.*

Chapter IV

Isabel Damas-Moreira, Julia L Riley, Miguel A Carretero, D James Harris, and Martin J Whiting. **Getting ahead: Exploitative competition by an invasive lizard.** *Manuscript in review.*

DIVISION OF WORK

Initials of each contributor is as follows:

IDM - Isabel Damas Moreira, **DJH** - David James Harris, **MJW** - Martin J Whiting,

AP - Ana Pereira, **DO** - Daniel Oliveira, **JLS** – Joana L Santos,

JR – Julia L Riley, **MAC** – Miguel A Carretero, **BP** – Bruno Pleno

| | I | II | III | IV |
|---------------------------|---------------------------|-----------------------------------|-----------------------|-------------------------------|
| Conception & Design | IDM, DJH, MJW | IDM, DJH, MJW | IDM, DJH, MJW | IDM, DJH, MAC, JLR, MJW |
| Planning & Implementation | IDM | IDM, DO | IDM, DJH | IDM |
| Data Collection | IDM, AP | IDM, DO, JS | IDM | IDM, BP* |
| Video Scoring | IDM | IDM | IDM | BP* |
| Data Analysis | IDM, JLR | IDM, JLR | IDM, JLR | IDM, JLR |
| Writing the manuscript | IDM | IDM | IDM | IDM |
| Paper Revisions | IDM, AP, JLR, DJH, MJW | IDM, DO, JLS, JLR, DJH, MJW | IDM, JLR, DJH, MJW | IDM, JLR, MAC, DJH, MJW |
| Overall Responsibility | IDM | IDM | IDM | IDM |

*BP assisted with data collection by scoring videos; not a co-author.

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"obrigada"

A PhD is a difficult time. It tests your skills, knowledge, and your ability to deal with challenges. In the end, it all culminates in a written piece of work that will always remind you how tough you can be. However, the PhD is a step in life that you do not take alone. It is mostly the support and knowledge of everyone else that makes you keep going. And for that, I am forever thankful.

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*Para ser grande, sê inteiro: nada
Teu exagera ou exclui.
Sê todo em cada coisa. Põe quanto és
No mínimo que fazes.*

*To be great, be whole:
Exclude nothing, exaggerate nothing that is not you.
Be whole in everything. Put all you are
Into the smallest thing you do.*

Fernando Pessoa, *in* Odes de Ricardo Reis, 1933.

ABSTRACT

The world has been changing at an unprecedented rate due to the ‘progress’ of human society. Globalization has changed our way of living, and it translates into severe negative effects on our ecosystems. Additionally, globalization also demands more connections and transport, creating frequent and dynamic networks worldwide, of both people and cargo. This can lead to the movement of thousands of species outside their native range, and biological invasions are now a contemporary global problem. While most invasions end in failure, some manage to successfully take hold and adapt to new locations. What determines a species' invasive success is of great interest and importance for conservation efforts. Behaviour is believed to play a key role in the success of invasive species, although the mechanisms are still unclear, especially for unintentional invasions. Members of the lizard genus *Podarcis* show high variability in their invasive potential and are thus a well-suited model for studying the role of behaviour during biological invasions. The Italian wall lizard, *Podarcis sicula*, is a globally invasive species that hitchhikes on transportation of people or cargo, and does well in novel environments. In addition to behaviour, there is mounting evidence that cognition may also be a determining factor for invasion success. The aim of my thesis is to understand the role of behaviour and cognition in determining what makes *P. sicula* such a good invader. I used animals from an introduced population in Lisbon (Portugal) to examine behavioural traits that might be linked to a species' invasive success.

My original contribution to knowledge is uncovering the potential role of behavioural flexibility, social learning ability, behavioural traits, and competition in the invasion process. Although I focused on the invasive lizard *P. sicula*, my study highlights the potential role of behaviour and cognition in invasions more broadly. My thesis has thus 4 chapters written as stand-alone publications that deal with different behavioural components. I predicted that *P. sicula* would have greater levels of behavioural flexibility than congeneric non-invasive species – *P. bocagei* and *P. carbonelli*. The ability to reverse a previously learnt discrimination can be indicative of behavioural flexibility. I used a discrimination task and a reversal and quantified the number of errors and overall learning ability of all three species (chapter I). The invasive species had relatively less difficulty than the non-invasive species to reverse the task. Also, I found different cognitive ability between the invasive species and

the two non-invasive species (*P. bocagei* and *P. carbonelli* had a more similar learning pattern between them). Chapter II dealt with the ability of *P. sicula* to obtain relevant social information to solve a task, from other *P. sicula*, or from a different species they had never encountered in nature (*P. bocagei*). The role of heterospecific learning in biological invasions has never been studied before. Remarkably, this invasive species learnt equally well from individuals of the same or different species. In chapter III, I used a sympatric congeneric species, *P. virescens*, native to the study site, to compare personality traits likely related to invasive success. I measured exploratory behaviour of lizards in a novel arena (activity and shelters visited); boldness (latency to emerge from a suboptimal shelter, after being scared into it); and neophobia (minimum distance between a lizard and a novel object placed in the arena). I found the invasive *P. sicula* to be more exploratory, neophilic, and bolder than the sympatric native species. Additionally, while the native species showed high repeatability in its behaviours and had all traits correlated, the invasive species was much less consistent and showed no correlation between traits. Finally, since the sympatric *P. virescens* has likely been displaced from gardens where *P. sicula* is found, interspecific competition could be an important factor governing their success as an invader. I thus assessed the mechanisms *P. sicula* might use to outcompete the sympatric *P. virescens* in this location (chapter IV). I established mixed groups of both species and scored several behaviours linked to competitive ability. Although I predicted competitive interference (e.g. the invasive species with more aggressive behaviours), I actually found evidence for competitive exploitation (e.g. the invasive species being more efficient at exploiting resources).

Collectively, my thesis shows differences in cognitive skills between invasive and non-invasive *Podarcis*, and that the invasive *P. sicula* is able to socially learn from the same and different species. Also, *P. sicula* has behavioural traits (e.g. boldness and foraging efficiency) that can be linked to its invasive success. The results from these chapters highlight the potential importance of behaviour in biological invasions, and give insight into why this lizard species is such a successful invader, and into what impact it might have on native species. This work will hopefully contribute to a better understanding of the behavioural basis of invasions, and ultimately assist conservation efforts.

INTRODUCTION

Globalization

The world is changing at an unprecedented rate due to the progress of human society (Tuomainen and Candolin 2011). Globalization has changed our way of living, but that also translates into severe negative effects on our ecosystems. The high levels of overpopulation, urbanisation, tourism, and all of its consequences (such as extreme land use, pesticides, deforestation, and climate change) has led to well-known impacts on ecosystems (Underwood et al 2009, Tuomainen and Candolin 2011). Additionally, globalization also demands more connections and transport, creating frequent and dynamic networks worldwide, of both people and cargo (Hulme 2009, Lewis et al 2016). This increase in mobility has influenced the distribution of life on earth, and currently, each day individuals of hundreds of species are unintentionally translocated outside their native range (Lockwood et al 2013, Chapple et al 2012). Consequently, over the last century, this has fuelled species introductions at a global scale (Vitousek et al 1996, Butchart et al 2010, Lewis et al 2016), and it is predicted the number of invasions will grow exponentially (Essl et al 2011). For example, by hiding in cargo, the brown tree snake (*Boiga irregularis*) has been taking rides in boats, and aircraft, and reaching distant locations, impacting these novel environments by causing mass power outages and causing the extinction of some native forest birds (Fritts and Rodda 1998, Lowe et al 2000). Nevertheless, perhaps the most famous case of an animal invader taking advantage of human activity to hitchhike and invade novel location is that of the rats. Rat species such as the black, *Rattus rattus*, or the brown rat, *Rattus norvegicus*, have historically been transported around the globe as accidental stowaways in ships, establishing themselves in many locations that were previously rat-free (Barnett 2001). They can severely impact and disrupt an ecosystem and have already caused the extinction of many small mammals, reptiles, and seabirds (Jones et al 2008, GISD 2011, 2014). These snake and rat species are considered among the top 100 most invasive species in the world (Lowe et al 2000). However, becoming an invasive species is not easy. A successful invader needs more than simply reach a new location, and most species fail to actually gain a foothold and establish themselves (Blackburn et al 2011).

Invasions

“Invasive species are that subset of alien species having a demonstrated negative effect on native ecosystems, species, or human values and concerns” (Kraus 2009). Invaders are a contemporary global problem, with major impacts at both economic and ecological levels (Pimentel et al 2000). Biological invasions are linked to half of modern extinctions (Clavero and García-Berthou 2005) and are one of the major causes involved in the current loss of biodiversity (Simberloff et al 2013). Nonetheless, becoming an invasive species involves overcoming several obstacles of the invasion process (Fig 1; Blackburn et al 2011, Chapple et al 2012, Griffin et al 2016), and the resulting invasive population is never a random subset of the source population; instead, it is made of the filtered individuals that managed to pass through all stages successfully (Blackburn et al 2011, Chapple et al 2012). Briefly, an invasion is a four-stage process: transportation, introduction, establishment, and invasion, with each stage incorporating different features and barriers, and challenging the organisms in different ways (Fig.1; reviewed in Colautti and MacIsaac 2004, Blackburn et al 2011 and Chapple et al 2012). Perhaps the most striking question in invasion biology is why are some species invasive and others are not? What makes some species able to conquer a new environment while others fail? Identification of these features is a central focus of invasion biology (Hayes and Barry 2007), and also crucial for adequate conservation efforts, as effective measures need detailed understanding of the mechanisms behind an invasion’s success (Holway and Suarez 1999). One explanation can be propagule pressure. This is based on the view that the more individuals that are introduced, the higher the probability of establishment and becoming invasive (Lockwood et al 2005, 2009, Simberloff 2009). However, while this might be partly true in some cases (Lockwood et al 2005, Hayes and Barry 2008, Simberloff 2009), it does not explain why some species repeatedly fail to invade new locations in initial high numbers, or why others become invasive with initial low numbers (Chapple et al 2012). Other relevant factors can also play a role in facilitating invasions, such as a similar environment between the native and introduced areas (Hayes and Barry 2007), enough resources in the new location (Kraus 2009), or the species’ life history traits (Capellini et al 2015). However, animal behaviour is often neglected as a missing piece in explaining the puzzle of what makes some species successful invaders while others are not. Adding a behavioural perspective to the study of biological invasions has hugely contributed

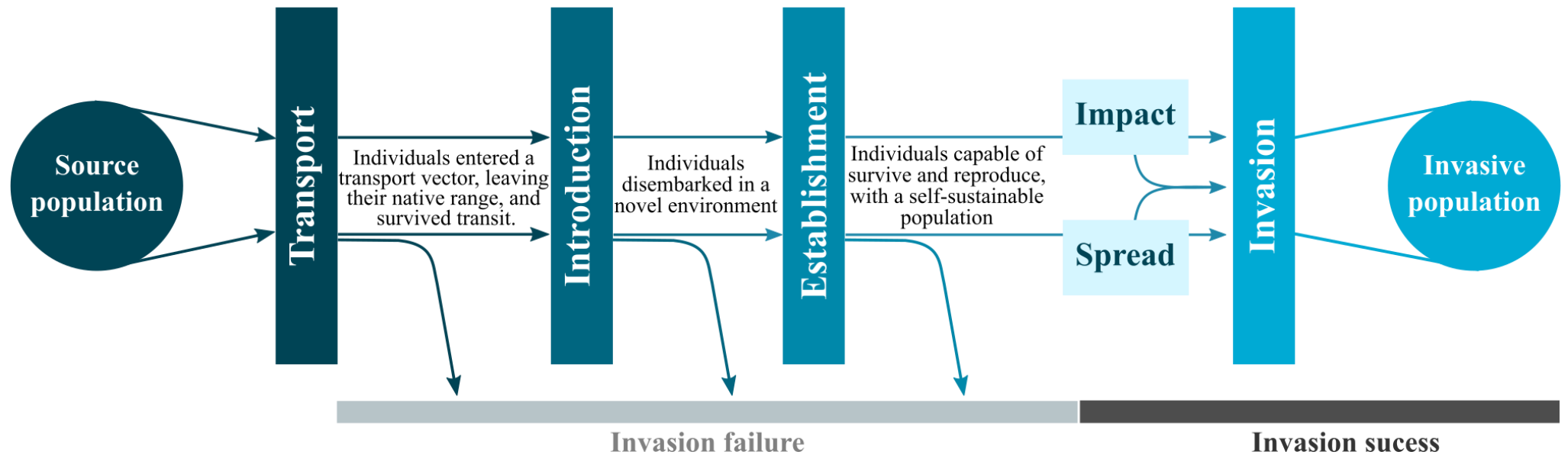


Fig 1. The invasive process. A species that fails at any stage of the process will not complete it and thus will not become invasive. The resulting invasive population consists of the filtered individuals that were able to overcome all obstacles in all stages successfully. Modified from Colautti and MacIsaac 2004, Blackburn et al 2011 and Chapple et al 2012, by IDM.

to the advancement of this field (Holway and Suarez 1999, Chapple et al 2012, Weis and Sol 2016). The exact role of behaviour in invasions is still unclear as generalizations for different contexts or for different species proved an extremely challenging task (Kraus 2009, Chapple et al 2012, Jeschke et al 2012, Phillips and Suarez 2012). It is important to address the behavioural mechanisms and consequences behind an invasion event in order to understand the general role of behaviour in invasions (Lodge 1993, Lewis et al 2016).

Behaviour in invasions

The invasion process includes different stages, each covering a different set of obstacles (Blackburn et al 2011). While some behaviours can be useful in different stages, others may be favourable in all stages of the invasion process (Chapple et al 2012), such as having high cognitive ability. Cognition involves the animal's perception, learning, memory, and its decision-making (Shettleworth 2001). Plausibly, a species that becomes invasive should have a remarkable ability to be cognitively flexible in order to adequately respond to changing conditions. The behavioural flexibility of an individual, i.e. the ability of an organism to rapidly adjust its behaviour to novel stimuli, is likely to increase the probability of a successful invasion (Sol et al 2002). Using brain size as an indirect proxy for behavioural flexibility, a link between larger brain size and establishment success has been found for different taxa, such as in invasive birds (Sol et al 2002, 2005), mammals (Sol et al 2008), and in amphibians and reptiles (Amiel et al 2011). Nevertheless, behavioural flexibility can also be directly measured by the ability of an animal to solve new tasks or existing problems in a novel way; through behavioural innovations or reversal of previously learnt acquisitions (Griffin et al 2013, Leal and Powel 2011, Griffin and Diquelou 2015). Being more flexible can help a species to better cope with novel habitats, predators, resources, and ecological situations (Sol et al 2002, Wright et al 2010, Chapple et al 2012), which can enhance the probability of reproduction and survival in a new environment (Sol et al 2002). Behavioural flexibility can also be correlated with other behavioural traits, which when combined, might increase the probability of invasion success (Sih et al 2012). For example, if a species has high levels of behavioural flexibility and can also employ social learning, it might be more likely to become established in a novel area (Wright et al 2010). Social learning occurs

when an individual is more likely to learn a new behaviour after observing another individual (Hoppitt and Laland 2008). This process can be a powerful way to obtain vital information that may improve an individual's fitness and survival. Hence, social learning occurs in a diverse array of taxa and contexts (Heyes 2012, Reader 2016). It can also be thought of as a shortcut to individual learning. Social learning can result in less risk and may be more economical than individual learning, in cases where an animal can learn the location of suitable shelters, food patches, which prey are dangerous, where to find water, or which locations to avoid (Gadef and Giraldeau 2001, Laland 2004, Heyes 2012).

Although this is rarely addressed, the combination between behavioural flexibility and social learning can lead to the rapid spread of innovative behaviours, advantageous in the novel location, through the population (Terkel 1995, Wright et al 2010, Aplin et al 2015). A good example that illustrates this point is that of the black rat, *Rattus rattus*, in Jerusalem (Terkel 1995). This species successfully invaded a pine forest because some individuals innovated a foraging technique and started to strip a pine cone to obtain the seeds. Remarkably, the new technique was then transmitted to the rest of the population by social learning (Terkel 1995). Since then, and although this is a powerful idea, there is surprisingly no studies, that I am aware of, on how social learning can influence the success of a species after reaching a new environment. Nevertheless, in environmental conditions varying unpredictably, as during an introduction event, the information from conspecifics can be unreliable as they were also recently translocated (Stephens 1991, Laland 2004, Wright et al 2010). Could it be that a species could thus learn from similar native species as well? The use of heterospecific learning occurs in nature across diverse contexts; different species can obtain cues from other species and learn anti-predator behaviours, where to find suitable habitat, or food sources (reviewed in Avarguès-Weber et al 2012). A species with the ability of acquiring new knowledge of innovative behaviour from a native species, could have a significant advantage during its introduction, especially when the introduced population comprises only few founder individuals, as commonly happens with human-assisted colonisations. Behavioural flexibility and social learning are thus two cognitive mechanisms that may play a major role during invasions. This is not surprising, given that cognition is being increasingly acknowledged for its role in fitness (Cole et al 2012, Thornton et al 2014), and should thus influence invasion success.

Besides addressing the importance of cognition in invasions, many other behavioural mechanisms could also enhance a species' invasive success (Chapple et al 2012). For example, introduction, establishment, and invasive success can be positively associated with attraction to and/or tolerance of human-occupied environments (Sol et al 2002, Kraus 2009), foraging behaviour (Weis 2010, Pintor et al 2009), neophilia (Candler and Bernal 2015, Griffin et al 2016), aggression (Weis 2010), activity, boldness, exploratory behaviour, and dispersal tendency (e.g. Cote et al 2010). If each one of these behaviours in an individual are consistent over time, it is termed animal personality (Réale et al 2007, Wolf and Weissing 2012). More complex than this, if these different behaviours are correlated, they form a behavioural syndrome (Chapple et al 2012, Sih et al 2004), and if such correlations promote invasions, then it is an invasion syndrome (Chapple et al 2012). Evidence is growing that some species are successful invaders because of correlated behaviours (Cote et al 2010, Michelangeli et al 2017, Pintor et al 2008, Rehage and Sih 2004). On the other hand, correlated traits might also constrain a population because if some factor impacts one behaviour it might also affect correlated ones (Sih et al 2012). Therefore, an invasive species could be more successful by presenting plastic behaviour and not always behaving in the same way. This could translate into greater success in novel environments (Dingemanse and Wolf 2013, Sih et al 2012). Moreover, particular behavioural traits that can promote the success of invasive species, can simultaneously affect native species. For example, having greater foraging efficiency can impact native species after introduction, by making less resources available for them (Pintor and Sih 2009, Short and Petren 2008). Consequently, this can displace or exclude native species from the introduced location, which can in turn reinforce the success of establishment and spread of the invasive species. Therefore, by studying the mechanism by which invasive species outcompete native species we can also obtain information on the behavioural traits that make it a successful invader. A more aggressive species can directly impact a native species through interference competition (Pintor and Sih 2009, Rowles and O'Dowd 2007), and a species with greater foraging efficiency can indirectly impact a native species through exploitative competition when resources are limited (Petren and Case 1996). Interestingly, an invasive species can employ both competition strategies to quickly displace native species and enhance its establishment and rate of spread (Human and Gordon 1996). Whatever strategy might be at play, it can result in severe impacts for native species and an advantage for the invasive species (Grether et al 2017).

The research within this thesis focuses on addressing the mechanisms and consequences of invasive success of a species that colonizes new locations through human-mediated dispersal. A recent study assessed the diversity and the quantity of herpetofauna that has been translocated by humans and found it to be much larger than previously believed, especially lizards (Chapple et al 2016). Most lizards might be able to avoid detection during transport by easily finding shelter in the complex structures of cargo (such as in ships, trucks, airplanes), for being small, and for having wide physiological tolerance (Kraus 2009, Chapple et al 2011). In order to assess how behavioural components can play a role in unintentional invasions (assisted by human vectors) the Italian Wall lizard *Podarcis sicula* is an excellent model.

Podarcis sicula

The Italian Wall lizard *Podarcis sicula* is a lacertid lizard native to the Italian Peninsula and Adriatic Coast, but invasive worldwide (Kraus 2009, Carretero and Silva-Rocha 2015). This species does not have one single population source, but instead, has multiple sources across its native range, to multiple introduced locations worldwide, which points to a general invasive ability of this species (Fig 2; Kolbe et al 2012, Podnar et al 2005, Silva-Rocha et al 2012, 2014). The main pathway for new introductions in the U.S.A. is due to intentional release or through the pet trade (Deichsel et al 2010; Kolbe et al 2012), while in Europe and the Mediterranean Basin it is due to *P. sicula* hitchhiking in the transportation of cargo or people (Silva-Rocha et al 2012, 2014, Carretero and Silva-Rocha, 2015, Mizsei et al 2016, Tok et al 2015, Molloy 2009). Also, this species can have an advantage during the transportation stage because it can be transported both through active or passive ways (Silva-Rocha et al 2014). This means lizards can both actively enter into a transport vector, or be transported simply because they were within a man-made structure that was being conveyed. *Podarcis sicula* often uses ornamental plants or olive trees, both of which are transported sometimes significant distances (Silva-Rocha et al 2014, Valdeón et al 2010, Rivera et al 2011). That the Italian wall lizard takes advantage of human-related movements is not a recent phenomenon. For example, in Menorca (Balearic islands, Spain), *P. sicula* was likely introduced during the Middle Ages due to intense trade with Italy (Mayol

1985, Pleguezuelos 2002). Interestingly, its invasion in Menorca occurred through multiple introduction events from lizards arriving from both Sardinia and Sicily (Silva-Rocha et al 2012).

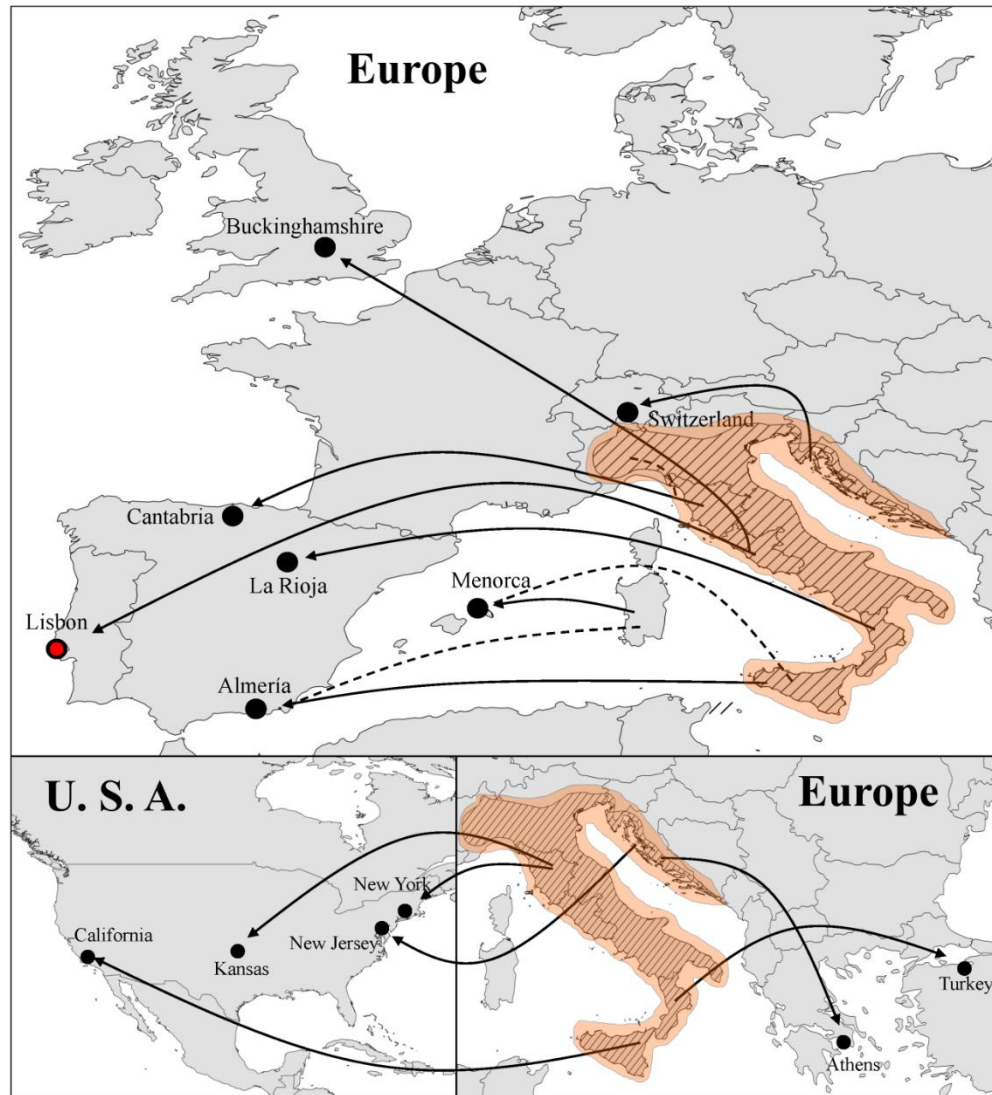


Fig 2. Examples of some pathways between source and introduced populations of *P. sicula*. The orange area with stripes indicates their native range. Arrows indicate the source and destination of introduced populations. The red dot indicates my study population. Adapted with permission from authors, Silva-Rocha et al 2014.

The Italian wall lizard have traits that give it a general advantage for human-assisted dispersal: they commonly inhabit human-occupied environments, and its distribution overlaps with multiple transport hubs (Hulme 2009, Chapple and Wong 2016). But so do other *Podarcis* species. Of 23 described species (Uetz et al 2018), the large majority of *Podarcis* species live near transport hubs but were never established outside their native range. Also, some common non-behavioural predictors for invasive species to be successful (such as propagule pressure and similarity between native and introduced ranges) seem to not adequately explain the success of *P. sicula*. Only a few individuals are necessary for the establishment of a population in a novel location (e.g. Vervust et al 2007, Herrel et al 2008). These lizards usually arrive in low numbers at new locations, and a controlled experiment revealed that only 5 pairs of *P. sicula* were able to successfully invade a novel location (Vervust et al 2007, 2010, Herrel et al 2008). The similarity between the environments in the native and introduced ranges also does not seem to explain the invasive success of all introduced populations. *Podarcis sicula* has previously been translocated to very different environments and managed to rapidly adapt (e.g. Burke et al 2002). Lizards that have been established in New York for a few decades (where winters can reach -20 °C), and initially coming from Rome (where winters can reach -7.4 °C), can burrow underground during hibernation and thereby survive the harsher winters (Burke et al 2002). The Italian wall lizard seems to adapt remarkably quickly. For example, a population introduced on a new island in Croatia was able to shift their diet to become herbivorous in < 35 years, eliminate the native *Podarcis melisellensis* from the island, and adapt its morphology, performance, and behaviour to the island conditions (Vervust et al 2007, 2010, Herrel et al 2008). Moreover, recently introduced populations appear to have high thermoregulation efficiency (study performed on a population introduced about a year before; Kapsalas et al 2016).

Established populations of Italian wall lizards have an impact on native species on the same trophic level (impacts on other biota are unknown). They can outcompete native species and displace them to poorer habitats (Nevo et al 1972, Downes and Bauwens 2002), exclude them (Nevo et al 1972, Capula et al 2002, Herrel et al 2008), and even hybridize with different *Podarcis* species (Capula 1993, 1994, 2002, Capula et al 2002). For example, the occurrence of this invasive species is one of the major threats to the critically endangered Aeolian Wall lizard *Podarcis raffonei* through competitive

exclusion and hybridization (Capula et al 2002), and was one of the causes for the disappearance of the native *Podarcis lilfordi* from the main island in Menorca (Pérez-Mellado 2002).

All evidence seems to indicate that behaviour can thus be key for *P. sicula* invasive success. The high variability in invasion potential between congeneric species in the genus *Podarcis* can present the opportunity to study behaviours that might be promoting invasion success. Such comparisons between invasive and non-invasive congeners can not only give insights on why particular species are successful invaders, but also help understanding potential impacts on native species (Carere and Gherardi 2013, Holway and Suarez 1999, Philips and Suarez 2012).

Study location

Podarcis sicula was introduced to Lisbon, Portugal, in 1998 (Fig 2), having arrived unintentionally from north Italy, likely with plant ornaments brought for the world exhibition that took place in Parque das Nações (Park of Nations) that year (González de la Vega et al 2001, Silva-Rocha et al 2012, CABI 2018). This park is extensive, and consists of modern buildings (offices, tourist attractions, stores, restaurants) and urban gardens. It is located by the river and has large numbers of visitors, since it is one of the major attractions for tourists visiting Lisbon. The Italian wall lizard inhabits urban gardens within this area (Fig 3). The species lives here at very high population density and is slowly expanding its range (Carretero and Silva-Rocha 2015). Portugal is home to several non-invasive small lacertids, including some *Podarcis* species (e.g. Fig 4), that may be impacted by the spread of *P. sicula*. The Italian wall lizard has already had an impact on at least one species, the native green Iberian wall lizard *Podarcis virescens*. It has likely displaced *P. virescens*, relegating this native species to surrounding gardens. Both species live in close sympatry, but not in syntopy (Ribeiro 2017).



Fig 3. The Italian wall lizard, *Podarcis sicula*, after collection from the wild in the urban gardens of Parque das Nações, Lisbon. This lizard was about 60 mm in snout-to-vent length. In the background is a completely urbanised landscape next to a garden of trees and bushes. Picture taken by IDM.

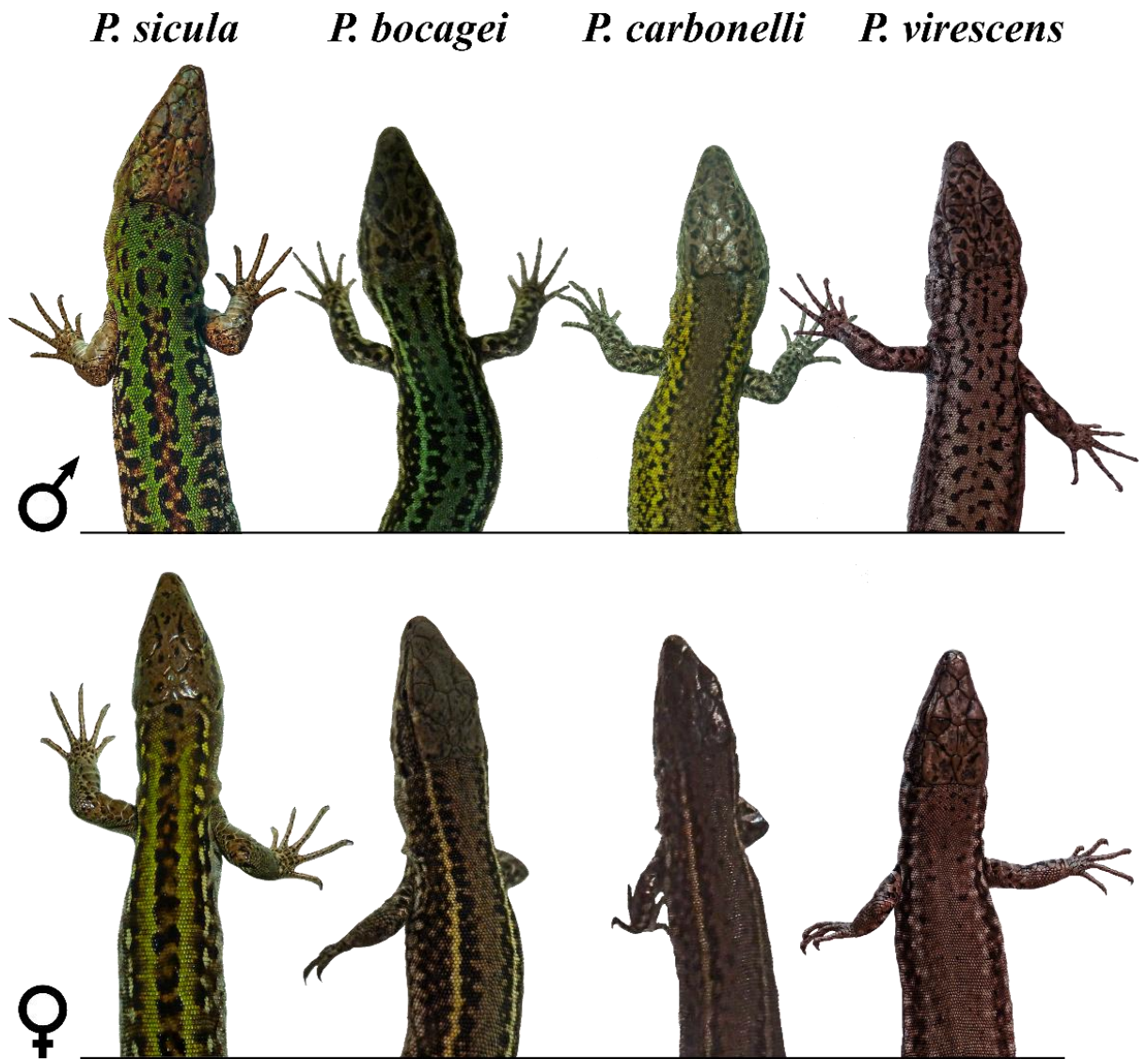


Fig 4. The *Podarcis* species used during the research of this thesis. Males are on the upper panel, and females on the lower. The focal species is the invasive *P. sicula* and was used in all four chapters. *Podarcis bocagei* was used in chapters I and II, *P. carbonelli* was used in chapter I, and *P. virescens* (the species living in sympatry with *P. sicula* in Lisbon) was used in chapters III and IV. All pictures taken by IDM.

AIMS OF THE STUDY

The main goal of this research thesis was to determine the behavioural mechanisms that make a species that reaches novel environments through human-mediated assistance, more likely to be a successful invader. Using a population of *Podarcis sicula* introduced in Lisbon, Portugal, in 1998, I examined the roles of cognition and behaviour in determining invasion success. The key questions I asked are listed for each chapter:

Chapter I: Is invasive potential linked to cognitive ability in a lizard?

Do invasive species have higher behavioural flexibility than non-invasive congeneric species?

Chapter II: Learning from others: An invasive lizard uses social information from both conspecifics and heterospecifics

Can an invasive species learn a novel foraging task more rapidly when social information is available from either a conspecific or heterospecific, compared to a control in which social information is absent?

Chapter III: Can behaviour explain invasion success? A comparison between sympatric invasive and native lizards

Do behavioural traits, such as exploration, neophobia, and boldness, differ between sympatric invasive and native species? Are the individuals of both species equally repeatable in their behavioural traits? Is there a correlation of these traits in both species?

Chapter IV: Getting ahead: Exploitative competition by an invasive lizard

Does an invasive species use interference (aggressive behaviours) or exploitative (dominates resources) competition to displace native species?

Each of the following chapters are written and formatted as stand-alone publications. Consequently, there is some unavoidable repetition. Moreover, different chapters might have slightly different formats because of the journal they were, or will be, submitted to.

CHAPTERS

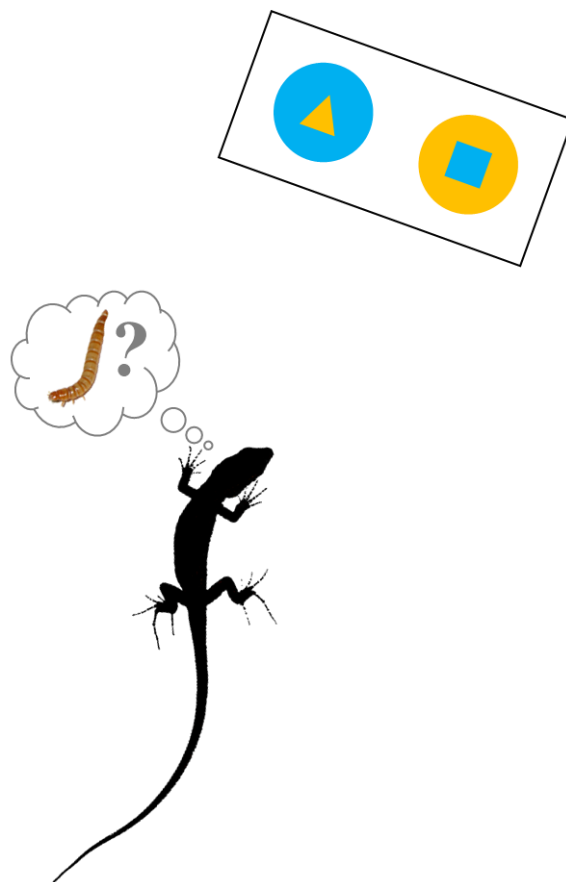


Chapter I

Is invasive potential linked to cognitive ability in a lizard?

Isabel Damas-Moreira, Ana Pereira, Julia L Riley, David J Harris, Martin J Whiting

Manuscript in preparation for submission



Is invasive potential linked to cognitive ability in a lizard?

ABSTRACT

Urbanisation is dramatically transforming the natural world and facilitating the introduction of numerous species into new areas. Nevertheless, only a few of these species become invasive. It is hypothesized that behavioural flexibility – the ability of an organism to rapidly adjust its behaviour to novel stimuli – can increase the probability of a successful invasion. Using a population of the invasive Italian wall lizard, *Podarcis sicula*, and two non-invasive congeners, *P. carbonelli* and *P. bocagei*, we tested for behavioural flexibility by measuring their ability to reverse a previously learned discrimination task between two cues (reward, no reward). We predicted that the invasive *P. sicula* would perform better at the reversal task (make fewer errors and reach learning criterion faster), and to have less difficulty in reversing the task compared to the discrimination task, than the two non-invasive congeneric species. Our results supported the second prediction, with the invasive species having less difficulty in reversing the task compared to the non-invasive species. While the native species had much more difficulty in reversing an acquired discrimination between two cues, the invasive species had a similar difficulty in acquiring the initial discrimination and in reversing it afterwards. This supports the hypothesis of moderately greater levels of behavioural flexibility in the invasive, relative to native, species. Importantly, the non-invasive species presented a similar pattern in their overall discrimination learning ability, which was different from the invasive species. Our results also highlight the importance of comparing closely related non-invasive species in order to better understand the behavioural mechanisms linked to the establishment of invasive species.

KEYWORDS

Podarcis sicula; invasion; behavioural flexibility; cognition

INTRODUCTION

Urbanisation is dramatically transforming the natural world and facilitating the introduction of species into new areas (Kraus 2009). While an increasing human population negatively impacts many species, some are able to expand their distribution range, aided by an increasing number of human transportation routes. Nevertheless, only a relatively small proportion of species are able to prosper in a new environment and become invasive (Blackburn et al 2011, Kraus 2009). A successful invasion is the product of many inter-related factors that include traits that might promote the success of the invasive species (Blackburn et al 2011, Chapple et al 2012). Often neglected, the behaviour of invasive species can increase our understanding of why some species are better than others at invading and colonizing novel locations (Holway and Suarez 1999, Chapple et al 2012). For many species, their successful establishment has been associated with higher aggression (Usio et al 2001, Rowles and O'Dowd 2007, Pintor et al 2008), foraging efficiency (Rehage et al 2005), boldness (Short and Petren 2008, Pintor et al 2008), and dispersal ability (Cote et al 2010, Rehage and Sih 2004). Nevertheless, few studies have addressed the role of cognitive ability in invasion success but offer insights into its plausible importance (Griffin et al 2016). For example, the invasive green crab *Carcinus maenas* has greater learning ability than the native blue crab *Callinectes sapidus* (Roudez et al 2008), and in crayfish, two invasive species – *Procambarus clarkia* and *Orconectes rusticus* – showed longer retention of learned associations than native crayfish species (Hazlett et al 2002). In birds, studies between invasive and non-invasive species demonstrate a link between higher rates of innovative feeding behaviour and establishment success (Sol et al 2002, 2005). These studies suggest invasive species have greater cognitive ability, which might ultimately indicate more behavioural flexibility compared to non-invasive species (Hazlett et al 2002, Sol et al 2002). Behavioural flexibility is the ability of an organism to rapidly adjust its behaviour to novel stimuli, by solving new problems or existing problems in a novel way (Leal and Powell 2011). Plausibly, this could be a key predictor of a species' success when adapting to a novel environment and facing new resources, habitat types, food, and predators (Sol et al 2002, 2008a, Wright et al 2010, Chapple et al 2012). In fact, an indirect link between behavioural flexibility (measured through brain size) and invasion success has been reported in mammals (Sol et al 2008b), birds (Sol et al 2002), and reptiles and amphibians (Amiel et al 2011). However, behavioural flexibility can also be measured directly, such as through problem solving

including associative learning, or reversal learning (Weis and Sol 2016). Comparing closely-related species with different invasion potential might offer insights into the role that learning ability plays during biological invasions (Sol et al 2002, Bezzina et al 2014). If closely-related non-invasive species exhibit lower levels of behavioural flexibility than invasive species, then this suggests a potential role for behavioural flexibility in determining invasive success. *Podarcis* lizards are a good model to test for differences in behavioural flexibility between closely related species because their invasive ability is variable. The genus *Podarcis* is found throughout the Mediterranean basin and currently includes 23 species (Uetz et al 2018). Interestingly, the Italian wall lizard *Podarcis sicula*, has been a particularly successful invader by colonizing new locations across the globe, with great efficiency (Kraus 2009, CABI 2018). This small lizard is native to the Italian Peninsula and the Adriatic coast, but has expanded its range to several other countries, from multiple origins in its native range (Silva-Rocha et al 2012, 2014, CABI 2018). Much of its range expansion is because of multiple long-range colonization events through anthropogenic transport corridors, resulting in this species occupying a wide variety of habitat types and environmental conditions (Carretero and Silva-Rocha 2015). Furthermore, the ability of *P. sicula* to rapidly adjust their morphology and behaviour to adapt to a new environment is quite remarkable. For example, one invasive population on a Croatian island experienced significant morphological change including the length of its digestive tract as it became more herbivorous after only 35 years (Vervust et al 2010, Herrel et al 2008). Another population that originated in Rome learnt to burrow underground to survive the harsher winters characteristic of New York City (Burke et al 2002). These behavioural adaptations hint at the potential importance of behavioural flexibility for this invasive species. Because these studies also indicate that these lizards benefit from changing their behaviour to increase their chances of survival in a novel environment, reversal learning can be an important mechanism through which to study behavioural flexibility. This can be quantified experimentally by measuring an individual's ability to reverse a previously learned discrimination task (Shettleworth 2010, Izquierdo et al 2016). This method has been applied to taxa as diverse as spiders (Liedtke and Schneider 2014), mammals (Chow et al 2017), fishes (Parker et al 2012, Lucon-Xiccato and Bisazza 2014) and lizards (Leal and Powell 2011, Riley et al 2018). In this type of experiment, an animal learns to discriminate between two different cues in which it associates one with a reward while the other is unrewarded; once this discrimination is acquired, the task is

reversed, switching rewarded and unrewarded cues. Differences in levels of behavioural flexibility can be measured between species by comparing the learning speed in the reversal task (number of errors and trials until acquisition); and, by comparing the relative difficulty in reversing the task compared to the discrimination task, as each species might have different initial acquisition times (Rajalakshmi and Jeeves 1965, Bond et al 2007, Day et al 1999). We examined behavioural flexibility between an introduced population of *P. sicula* in Lisbon (Portugal) and two non-invasive *Podarcis* species, Carbonell's wall lizard (*P. carbonelli*), and Bocage's wall lizard (*P. bocagei*). Both *P. carbonelli* and *P. bocagei* can also be found in Portugal and have relatively small distribution ranges. As far as we know, these two species have never been observed outside their native distribution, although it is likely they have had the opportunity to leave their native range as both live across urbanised areas and transport hubs within their distribution. We predicted that the invasive Italian wall lizard would be better at solving a novel foraging task. Specifically, we tested two predictions: that the invasive *P. sicula* 1) would perform better within the reversal task (make fewer errors on the reversal task and reach learning criterion faster), and 2) would have less relative difficulty in reversing the task, compared to the initial discrimination task, than the two non-invasive congeneric species.

MATERIAL AND METHODS

Study lizards and locations

In total, we collected 60 adult lizards of three species from the wild: *Podarcis sicula*, *P. carbonelli*, and *P. bocagei* (20 of each species; 10 of each sex). *Podarcis sicula* were collected from Parque das Nações, Lisbon, West Portugal (N 38° 45' 43.8", W 9° 5' 41.7"). This population is genetically assigned to the Italian Tuscany region (Silva-Rocha et al 2012), and was likely introduced in 1998 during the world EXPO 98 exhibition, after being brought in with ornamental plants (González de la Vega et al 2001). *P. carbonelli* was collected in Torreira, Northwest Portugal (N 40° 45' 49.1", W 8° 42' 39.7"), which is within their small native range that extends through the central Iberian mountain system and southwest coastal regions. *P. bocagei* was collected in Vairão, Northwest Portugal (N 41° 18' 37.6", W 8° 40' 32.1"), which is also within their limited range in the northwest Iberian Peninsula. All three collected populations occupy urban habitats.

Lizards were captured and transported to the lab at CIBIO-InBIO, University of Porto, on the same day, and all had a complete, or a fully-regenerated, tail. During their time in captivity they were housed individually in tubs measuring 200 mm W \times 300 mm L \times 200 mm H, maintained on a 12:12 h light:dark photoperiod and provided with a refuge and water *ad libitum*. The room temperature was set at 26°C during the day, a common temperature for maintaining *Podarcis* lizards in captivity (e.g., *P. sicula*; Bonacci et al 2008).

Experimental protocol

Lizards were first trained to extract food rewards from the experimental apparatus. In the first training phase they were fed from a transparent, open petri dish. In the second training phase the petri dish was opaque (black tape); and in the final training phase the opaque petri dish was half covered by a red lid. Lizards moved to the next training phase after eating food 4/5 consecutive times. After this training phase, all animals were presented three tasks in the following order: motor, discrimination, and reversal. We conducted learning trials twice a day, for five days before giving them a two-day break to avoid trial fatigue. Each trial was an hour in duration and was separated from the previous trial by at least 1 hour. In all tasks, the food reward was a live mealworm. Each petri dish was 3.5 cm diameter and each lid was 4.5 cm diameter, allowing a 0.5 cm lip.

In the motor task, lizards had to remove a red lid that completely covered the petri dish in order to access the food reward. A trial was scored as successful if the lizard removed the lid, and unsuccessful if the lizard touched the dish or lid but did not open it. Trials in which the lizard did not engage with the apparatus were excluded from analysis. In the discrimination task, lizards were presented with two dishes covered by either a yellow (reward) or blue (non-reward) lid. The reversal task was similar to the discrimination task but with opposite cues (blue: reward, yellow: non-reward). We incorporated a shape of the opposite colour in the lids (a blue square on the yellow lid and a yellow triangle on the blue lid) to provide them two cues to discriminate the lids (colour and shape), and to attempt to control for colour avoidance or preference. For both tasks, the trial was considered successful if the lizard removed the lid from the reward dish first, and unsuccessful if it removed the non-reward lid first. The trial was not included in the analysis if the lizard did not remove any lid, interact with the

experimental apparatus, or eat. A mealworm was placed in both dishes to control for odour and auditory cues. However, in the reward dish the mealworm was accessible while in the non-reward dish the mealworm was below mesh fixed with putty and therefore inaccessible. Mesh and putty were also placed in the reward dish, but in a way that made the mealworm still accessible. Dishes and lids were cleaned with cleaning wipes at the end of each trial, and randomly redistributed in the next trial. The position of the cue reward was randomized (left or right) between each trial and lizard. We considered lizards to have learnt a task when they were successful in 7/7 or 7/8 consecutive trials (Riley et al 2018), at which point they would move on to the next task. If after 40 trials the learning criterion was not reached, we removed the lizard from the experiment (Fig 1). All trials were remotely video-recorded with CCTV cameras, and later scored by IDM.

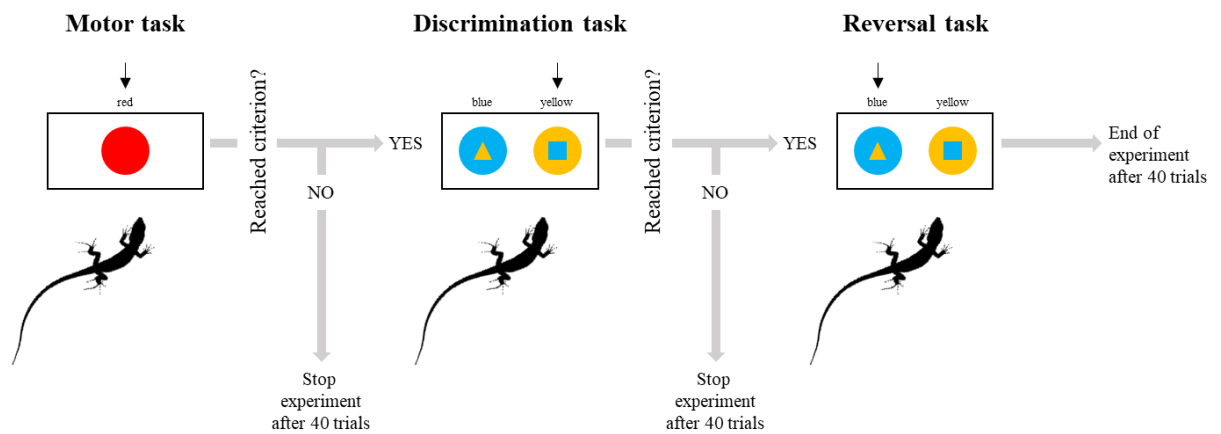


Fig 1. Schematic representation of the tasks, and their sequence. Each lizard could only move to the next stage after reaching the learning criterion in that task. The black arrows indicate the reward dish.

Statistical analysis

From the videos, we scored the lizards' choice in each trial (correct (1) or incorrect (0)), and the latency (in seconds) between the moment the experimental apparatus was inserted in the terrarium until the lizard opened the correct dish. We obtained five response variables from this data: a) number of trials until reaching criterion; b) proportion of correct choices within a task (i.e. the number of correct choices over the total number of trials the lizard carried out in each task); c) probability of

making a correct choice within a trial; d) latency to open the reward dish; and e) the number of lizards that learnt.

First, we analysed the data separately for each task – motor, discrimination, and reversal – to test for significant differences in the performance of each species within each task. However, the response variables in each of the models were consistently the same. Because all animals learnt the motor and discrimination task, we only analysed differences in the number of lizards that learnt the reversal task.

All statistical analyses were performed in R version 3.4.2 (R Core Team 2017) and before all analyses, we used the protocol from Zuur et al (2010) to explore our data and ensure it fitted model assumptions (i.e., variable distributions, reviewing data integrity, no influential outliers, no collinearity between predictor variable within a model, etc.). We used linear mixed effect models (LMM) with the function *lmer* in the *lme4* package (Bates et al 2015); and generalized linear models (GLM). We also used generalized linear mixed effect models (GLMMs) with the function *glmer* in the *lme4* R package (Bates et al 2015) to analyse our data when there was non-independence in the data due to repeated measures of the same individual. For the three tasks individually, we performed the same analysis:

- a) A Poisson GLM examined if the number of trials until criterion was influenced by species (*P. sicula*, *P. bocagei*, or *P. carbonelli*) and sex (male or female). We also accounted for the interaction between species and sex, and removed them from the final model if they were non-significant.
- b) A binomial GLM with a binomial distribution (logit link; Crawley 2012) examined if the proportion of correct choices (ranging from 0 to 1) was influenced by species and sex, with an additional interaction of species and sex, which was removed if not significant.
- c) A binomial GLMM examined if the probability of making a correct choice within a trial (correct choice in the trial = 1, incorrect choice in the trial = 0) was influenced by the trial number, species or sex. We also included a random intercept and slope for lizard identity across trials due to the dependency among repeated observations of the same individual. This model also included an interaction between trial number and species that was later removed if not significant.

- d) A Gaussian LMM examined if latency until the correct choice (time in seconds) was influenced by trial number, species or sex. As in c), we also incorporated a random intercept and slope for lizard identity across trials due to repeated observations. In the motor task, we incorporated an interaction trial \times species \times sex, which was removed if not significant. We did not need to account for any interactions for the other two tasks.
- e) For the reversal task, we used a binomial GLM to analyse if the number of lizards that learnt the task (learnt = 1, not learnt = 0) was affected by species or sex. We also included in the model an interaction between species and sex, which was removed if non-significant.

To test for significant differences between all contrasts, we used the *lsmeans* R package with the function *lsmeans* (Lenth 2016). All *P*-values were corrected using Tukey's HSD multiplicity adjustment (Lenth 2016). For all models, α was set at 0.05. We always examined the assumptions of all models. When we predicted fitted lines from the models for visualization, we set the factorial fixed factors to intercept-level values.

Second, we combined datasets from all tasks, to test differences between the performances of each species across the different tasks. For this dataset, we had to use GLMMs due to repeated measures for the same individual (given the same individual participated in the three tasks). To investigate differences in the number of trials until criterion and in the proportion of correct choices, we used GLMMs with the distributions as specified above for the respective variable. We included a random effect of lizard identity, and the fixed effects of species, sex, and task. To investigate the probability of making a correct choice and the latency, we used the modelling approach described above, but with the new fixed effect of task. For all these four models, we included an interaction between species and task, and used *lsmeans* as above, to analyse the contrasts between these predictor variables (both factorial).

RESULTS

Motor task

All lizards opened the dishes with their snout, and all learnt the motor task (Table 1). However, *P. carbonelli* took significantly more trials to reach acquisition compared to *P. bocagei* and *P. sicula* ($\beta = 0.261$, z -value = 2.438, $P = 0.015$; Fig 2, Table S1a, Table S2a), but there were no significant differences between *P. bocagei* and *P. sicula*, nor between the sexes (Table S2a). Species and sex did not have a significant effect on the proportion of correct choices (Table S1b and Table S2b). In this task, the probability of making a correct choice increased with trials ($\beta = 0.724$, z -value = 3.050, $P = 0.002$, Table S1c) and males had a higher probability of making a correct choice than females ($\beta = 1.229$, z -value = 2.472, $P = 0.013$, Table S1c). Also, *P. bocagei* and *P. sicula* were more likely to make a correct choice than *P. carbonelli* ($\beta = -2.022$, z -value = -3.435, $P = 0.001$, Table S1c and Table S2c). Latency decreased across trials ($\beta = -35.030$, z -value = -2.550, $P = 0.011$, Table S1d) but was not significantly different between species (Table S2d).

Discrimination task

All lizards learnt this task (Table 1), and no sex effects were detected in any response variable (Table S1). *Podarcis sicula* required significantly more trials to reach criterion than the other two species ($\beta = 0.449$, z -value = 2.993, $P = 0.003$, Fig. 2, Table S1a, Table S2a); while *P. carbonelli* and *P. bocagei* were not significantly different (Table S1a, Table S2a). As in the motor task, we did not detect any effect of species or sex on the proportion of correct choices (Table S1b, Table S2b). *Podarcis sicula* also had a lower probability of making a correct choice compared to the other two species ($\beta = -0.600$, z -value = -2.993, $P = 0.003$), while *P. carbonelli* and *P. bocagei* were again not significantly different (Table S1c, Table S2c). *Podarcis carbonelli* had a longer latency compared to *P. sicula* and *P. bocagei* ($\beta = 242.339$, z -value = 4.144, $P < 0.0001$, Table S1d, Table S2d).

Reversal task

We detected no sex effects in any of the response variables for this task (Table S1) and this was the only task in which not all lizards attained our learning criterion – 80% of *P. sicula*, 70% of *P. bocagei*, and 45% of *P. carbonelli* acquired the reversal (Table 1). However, the number of learners did not differ significantly between species (Table S1e, Table S2e), nor did the number of trials to reach criterion (Table S1a, Table S2a). In fact, we found no effect from species or sex in those two response variables (Table S1a and e, Table S2a and e). In addition, as in the motor and discrimination tasks, species and sex did not have a significant effect on the proportion of correct choices (Table S1b) and Table S2b). The probability of making a correct choice increased across trials during the reversal task ($\beta = 0.057$, z -value = 6.319, $P < 0.0001$, Table S1c) and *P. sicula* had a higher probability of making a correct choice than the other two species ($\beta = 0.533$, z -value = 2.945, $P = 0.003$), with no significant differences between *P. bocagei* and *P. carbonelli* (Table S1c, Table S2c). Latency decreased across trials ($\beta = -8.943$, z -value = -3.913, $P < 0.0001$, Table S1d). Latency between *P. sicula* and *P. bocagei* was not significantly different, but *P. carbonelli* was significantly slower than the other two species ($\beta = 189.124$, z -value = 3.851, $P < 0.0001$, Table S1d, Table S2d). For all values regarding the three tasks, please see Table S1 and S2 in the supplementary material.

Table 1. Sample sizes and mean values for the number of trials until criterion (Number of trials), and for the proportion of correct choices (Proportion, from 0 to 1), for each task. The total number of animals tested (N) is indicated for each species and sex. N_{learners} refers to the number of lizards that reached learning criterion for each task (out of total 20 animals for each species). In the reversal task, only the lizards that learned the task were included in the mean values of Number of trials to reach criterion, but all 60 lizards were included in the mean values of the proportion of correct choices. For all mean values, the standard error (SE) follows the means.

| Species | Sex | N | MOTOR TASK | | | DISCRIMINATION TASK | | | REVERSAL TASK | | |
|----------------------|-------|----|-----------------------|------------------|---------------|-----------------------|------------------|---------------|-----------------------|------------------|---------------|
| | | | N _{learners} | Number of trials | Proportion | N _{learners} | Number of trials | Proportion | N _{learners} | Number of trials | Proportion |
| <i>P. carbonelli</i> | M | 10 | 10 | 9.2 (± 1.0) | 0.88 (± 0.05) | 10 | 11.8 (± 2.1) | 0.82 (± 0.06) | 5 | 22.8 (± 1.56) | 0.49 (± 0.04) |
| | F | 10 | 10 | 10.8 (± 1.5) | 0.84 (± 0.06) | 10 | 11.3 (± 2.1) | 0.85 (± 0.05) | 4 | 26.5 (± 3.01) | 0.47 (± 0.04) |
| | M + F | 20 | 20 | 10.0 (± 0.9) | 0.86 (± 0.04) | 20 | 11.6 (± 1.4) | 0.83 (± 0.04) | 9 | 24.4 (± 1.6) | 0.48 (± 0.03) |
| <i>P. bocagei</i> | M | 10 | 10 | 7.0 (± 0.0) | 1.00 (± 0.00) | 10 | 12.6 (± 2.5) | 0.79 (± 0.04) | 8 | 27.3 (± 2.86) | 0.50 (± 0.04) |
| | F | 10 | 10 | 8.4 (± 1.0) | 0.91 (± 0.05) | 10 | 10.9 (± 1.3) | 0.85 (± 0.04) | 6 | 21.7 (± 2.26) | 0.49 (± 0.04) |
| | M + F | 20 | 20 | 7.7 (± 0.5) | 0.96 (± 0.03) | 20 | 11.8 (± 1.4) | 0.82 (± 0.03) | 14 | 24.9 (± 2.0) | 0.49 (± 0.03) |
| <i>P. sicula</i> | M | 10 | 10 | 7.2 (± 0.1) | 0.98 (± 0.02) | 10 | 16.2 (± 2.9) | 0.74 (± 0.04) | 7 | 22.1 (± 3.7) | 0.58 (± 0.04) |
| | F | 10 | 10 | 7.6 (± 0.4) | 0.95 (± 0.03) | 10 | 20.7 (± 3.1) | 0.71 (± 0.04) | 9 | 20.2 (± 1.99) | 0.59 (± 0.04) |
| | M + F | 20 | 20 | 7.4 (± 0.2) | 0.96 (± 0.02) | 20 | 18.5 (± 2.1) | 0.73 (± 0.03) | 16 | 21.1 (± 1.9) | 0.58 (± 0.03) |

Comparison between species across tasks (combined dataset)

All 60 lizards learned the motor and discrimination tasks, but not all reached the learning criterion in the reversal (Table 1). Importantly, we found no effect of sex in any response variable (Table 2, a - d).

The number of trials taken to reach learning criterion were significantly different between the species across the tasks (Table 2a, Table 3a, Fig 2). Both *P. carbonelli* and *P. bocagei* needed significantly more trials in the reversal task compared to both the motor and discrimination tasks (Table 3a).

Podarcis bocagei also needed more trials in the discrimination than in the motor task, but not *P. carbonelli* (Table 3a). *Podarcis sicula* took significantly more trials in the discrimination and reversal tasks compared to the motor task, but there were no differences in the number of trials taken to learn between the discrimination and reversal tasks (Table 3a).

We found an effect of the reversal task on the proportion of correct choices each species made (Table 2b). *Podarcis carbonelli* and *P. bocagei* had the same proportion of correct choices in the discrimination and motor tasks, but both made significantly more errors in the reversal than in the motor and discrimination tasks (Table 3b). *Podarcis sicula* made a higher proportion of correct choices in the motor than in the reversal task, but the motor and discrimination tasks were not significantly different, nor were the discrimination and reversal tasks (Table 3b).

The probability of making a correct choice increased across trials (Table 2c). The three species had a significantly higher probability of making a correct choice in the motor and in the discrimination than in the reversal task (Table 3c). *Podarcis bocagei* and *P. sicula* also had a higher probability of making a correct choice in the motor compared to the discrimination task (Table 3c).

Lastly, latency decreased across trials (Table 2d) with a significant effect from *P. carbonelli*, *P. sicula* and motor task (Table 2d). Nevertheless, all three species exhibited no significant differences in their latency between the different tasks (Table 3d).

Table 2. Outcome of the linear mixed effect models (GLMM) examining the effect of species (*P. sicula*: PS, *P. bocagei*: PB, or *P. carbonelli*: PC), sex (males: M, females: F), and task (motor: MOT, discrimination: DIS, reversal: REV) on the four response variables in study (a - d). Reference levels for all fixed factors are shown in parentheses. Indicated below each response variable is the total number of observations (N_{obs}). For all four response variables, N_{task}=3 and N_{individuals}=60. When the model did not include the fixed factor “trials”, this is indicated with a dash (-). All significant results are presented in bold.

| | a) Number of trials to reach criterion | | | | b) Proportion of correct choices | | | | c) Probability of making a correct choice | | | | d) Latency during trials | | | |
|---------------------------|--|---------------|---------------|------------------|----------------------------------|--------------|---------------|------------------|---|--------------|---------------|------------------|--------------------------|---------------|---------------|------------------|
| | N _{obs} =159 | | | | N _{obs} =180 | | | | N _{obs} =3082 | | | | N _{obs} =3082 | | | |
| | β | SE | z-value | P | β | SE | z-value | P | β | SE | z-value | P | β | SE | z-value | P |
| Intercept (PB, F, DIS) | 2.460 | 0.088 | 28.014 | <.0001 | -0.249 | 0.103 | -2.415 | 0.016 | 0.987 | 0.178 | 5.555 | <.0001 | 464.378 | 41.436 | 11.207 | <.0001 |
| Trials | - | - | - | - | - | - | - | - | 0.045 | 0.007 | 6.136 | <.0001 | -7.123 | 2.265 | -3.144 | 0.002 |
| Species (PC) | -0.016 | 0.114 | -0.142 | 0.887 | -0.027 | 0.140 | -0.190 | 0.849 | -0.117 | 0.231 | -0.508 | 0.611 | 237.695 | 53.434 | 4.448 | <.0001 |
| Species (PS) | 0.446 | 0.107 | 4.176 | <.0001 | -0.137 | 0.128 | -1.069 | 0.285 | -0.6701 | 0.209 | -3.209 | 0.001 | 128.157 | 51.938 | 2.467 | 0.014 |
| Sex (Male) | -0.035 | 0.070 | -0.502 | 0.616 | 0.018 | 0.059 | 0.311 | 0.756 | 0.065 | 0.106 | 0.610 | 0.541 | 16.177 | 29.847 | 0.542 | 0.588 |
| Task (MOT) | -0.423 | 0.103 | -4.087 | <.0001 | 0.166 | 0.152 | 1.092 | 0.275 | 1.345 | 0.353 | 3.811 | <.0001 | 122.515 | 57.429 | 2.133 | 0.033 |
| Task (REV) | 0.779 | 0.088 | 8.879 | <.0001 | -0.528 | 0.123 | -4.305 | <.0001 | -1.821 | 0.195 | -9.362 | <.0001 | 68.538 | 45.542 | 1.505 | 0.132 |
| Species (PC) : Task (MOT) | 0.279 | 0.141 | 1.971 | 0.049 | -0.110 | 0.210 | -0.522 | 0.602 | -1.242 | 0.423 | -2.933 | 0.003 | 12.119 | 80.114 | 0.151 | 0.880 |
| Species (PS) : Task (MOT) | -0.491 | 0.142 | -3.461 | 0.001 | 0.162 | 0.209 | 0.775 | 0.438 | 0.989 | 0.521 | 1.897 | 0.058 | -16.265 | 79.249 | -0.205 | 0.837 |
| Species (PC) : Task (REV) | -0.030 | 0.134 | -0.223 | 0.824 | 0.039 | 0.172 | 0.225 | 0.822 | 0.161 | 0.264 | 0.612 | 0.541 | -62.416 | 62.973 | -0.991 | 0.322 |
| Species (PC) : Task (REV) | -0.661 | 0.1167 | -5.662 | <.0001 | 0.300 | 0.1656 | 1.814 | 0.070 | 1.154 | 0.246 | 4.687 | <.0001 | -92.983 | 60.052 | -1.548 | 0.122 |

Table 3. Relevant results from the pairwise comparisons between the species across tasks for the four response variables in study (a - d). Indicated below each response variable is the total number observations (N_{obs}). For all four response variables, $N_{\text{task}}=3$ and $N_{\text{individuals}}=60$. All significant results are presented in bold.

| Species | Contrast | a) Number of trials to reach criterion | | | | b) Proportion of correct choices | | | | c) Probability of making a correct choice | | | | d) Latency during trials | | | |
|----------------------|---------------------------------|--|--------------|------------------|-------------------|----------------------------------|--------------|------------------|-------------------|---|--------------|------------------|-------------------|--------------------------|---------|------------------|-------------------|
| | | $N_{\text{obs}}=159$ | | | | $N_{\text{obs}}=180$ | | | | $N_{\text{obs}}=3082$ | | | | $N_{\text{obs}}=3082$ | | | |
| | | β | SE | $z\text{-value}$ | P_{corr} | β | SE | $z\text{-value}$ | P_{corr} | β | SE | $z\text{-value}$ | P_{corr} | β | SE | $z\text{-value}$ | P_{corr} |
| <i>P. carbonelli</i> | Discrimination - Motor Tasks | 0.144 | 0.096 | 1.496 | 0.858 | -0.056 | 0.145 | -0.387 | 1.00 | -0.103 | 0.234 | -0.442 | 1.000 | -134.633 | 55.853 | -2.410 | 0.278 |
| | Discrimination - Reversal Tasks | -0.749 | 0.101 | -7.411 | <.0001 | 0.489 | 0.121 | 4.030 | 0.002 | 1.660 | 0.188 | 8.835 | <.0001 | -6.122 | 44.314 | -0.138 | 1.000 |
| | Motor - Reversal Tasks | -0.893 | 0.104 | -8.561 | <.0001 | 0.545 | 0.126 | 4.321 | 0.001 | 1.763 | 0.203 | 8.688 | <.0001 | 128.511 | 51.305 | 2.505 | 0.229 |
| <i>P. bocagei</i> | Discrimination - Motor Tasks | 0.422 | 0.103 | 4.087 | 0.001 | -0.166 | 0.152 | -1.092 | 0.9756 | -1.345 | 0.353 | -3.811 | 0.004 | -122.515 | 57.429 | -2.133 | 0.450 |
| | Discrimination - Reversal Tasks | -0.779 | 0.088 | -8.879 | <.0001 | 0.528 | 0.123 | 4.305 | 0.001 | 1.821 | 0.195 | 9.362 | <.0001 | -68.538 | 45.542 | -1.505 | 0.854 |
| | Motor - Reversal Tasks | -1.202 | 0.100 | -12.062 | <.0001 | 0.694 | 0.137 | 5.053 | <.0001 | 3.167 | 0.335 | 9.463 | <.0001 | 53.977 | 54.237 | 0.995 | 0.986 |
| <i>P. sicula</i> | Discrimination - Motor Tasks | 0.914 | 0.097 | 9.412 | <.0001 | -0.328 | 0.143 | -2.290 | 0.348 | -2.334 | 0.384 | -6.076 | <.0001 | -106.250 | 54.759 | -1.940 | 0.586 |
| | Discrimination - Reversal Tasks | -0.118 | 0.077 | -1.543 | 0.835 | 0.227 | 0.111 | 2.042 | 0.514 | 0.667 | 0.154 | 4.347 | 0.001 | 24.445 | 39.415 | 0.620 | 1.000 |
| | Motor - Reversal Tasks | -1.032 | 0.100 | -10.362 | <.0001 | 0.555 | 0.140 | 3.972 | 0.002 | 3.002 | 0.380 | 7.889 | <.0001 | 130.695 | 54.0264 | 2.419 | 0.273 |

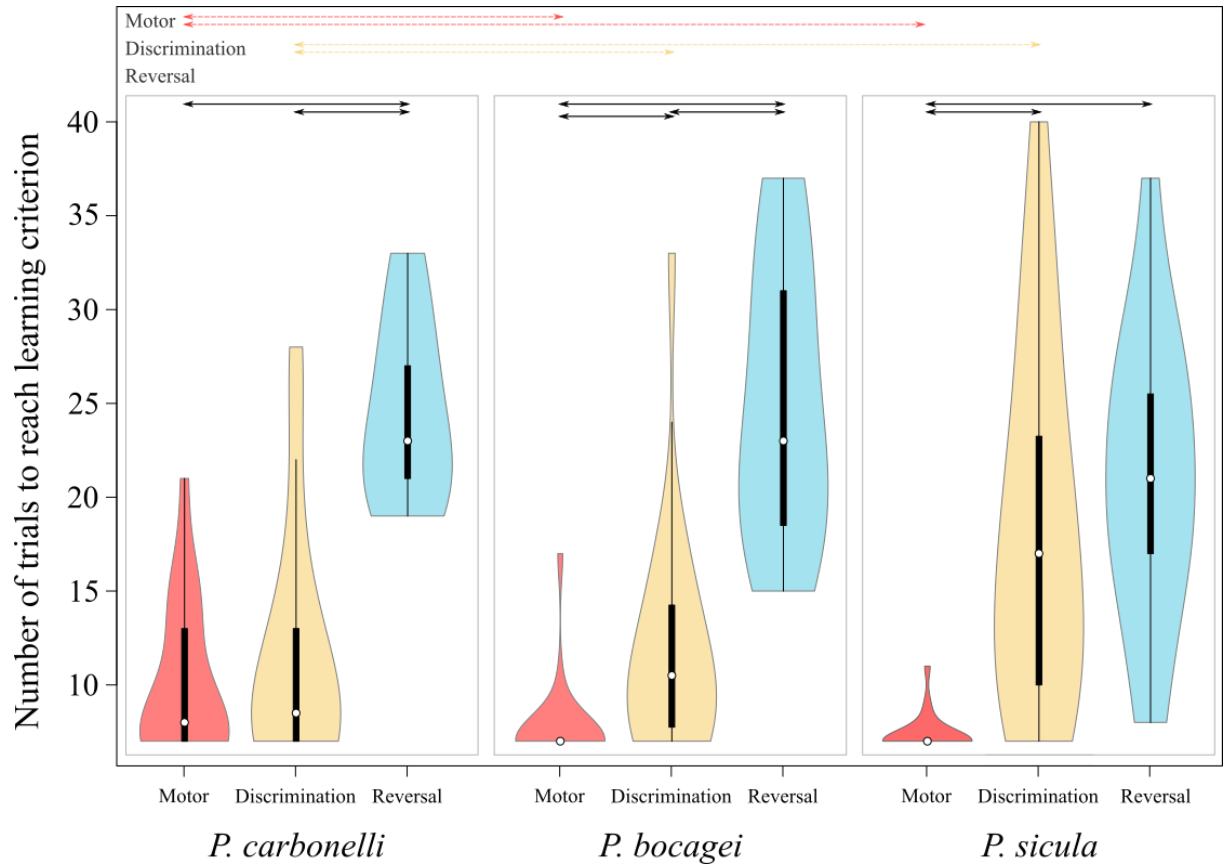


Fig 2. Violin plots showing the number of trials needed to reach the learning criterion within each task and species. The minimum number of trials to reach criterion is 7. For each species, the plot is coloured to correspond with the reward colour of the task. In each plot, the white dot represents the median, the thick black bar the 1st to the 3rd quartiles, the black thin line the minimum and maximum, and the coloured area represents a data density plot to the right and left of the line. Arrows represent the significant differences – black solid arrows within each plot show the differences between tasks for the same species, while the dashed coloured arrows outside the plots show the differences between species for the same task. There are no arrows for the reversal task because there were no significant differences between species in that task.

DISCUSSION

We tested for behavioural flexibility in the invasive *P. sicula* and two non-invasive congeners, *P. bocagei* and *P. carbonelli*, through a reversal learning task. We found moderate evidence for higher levels of behavioural flexibility in the invasive Italian wall lizard. All individuals learnt the motor task, but *P. carbonelli* took significantly more trials to learn it. However, on average *P. bocagei* and *P. sicula* were only 2.3 and 2.6 trials faster, respectively (these two species were not significantly different). Again, all lizards learnt the discrimination task, but the invasive *P. sicula* performed the worst, taking significantly more trials to acquisition compared to the two non-invasive species. While in the motor and discrimination tasks all lizards were able to reach acquisition, not all lizards learnt the reversal task. The number of successful learners did not significantly differ between species (80% of *P. sicula*, 70% of *P. bocagei*, and 45% of *P. carbonelli* learnt the reversal within the cut-off of 40 trials), but this may be due to low statistical power. Contrary to what we expected, all three species performed similarly at the reversal task – the number of trials taken to learn and the proportion of correct choices were not significantly different. Even so, *P. sicula* had the highest probability of making a correct choice in a trial during reversals. As predicted, across tasks *P. sicula* had less relative difficulty in reversing the discrimination than the other two non-invasive species. While *P. carbonelli* and *P. bocagei* performed significantly worse at the reversal compared to the discrimination task (needed more trials to learn and made more mistakes in the reversal than discrimination), *P. sicula* performed similarly between both tasks.

Our cognitive tests did not reveal a high level of behavioural flexibility in *P. sicula*, but the degree of behavioural flexibility they exhibited was still higher than in the two non-invasive species. This is because *P. sicula* performed the best at reversal learning relative to discrimination (same number of trials, amount of errors and higher proportion of lizards reached acquisition). Learning a reversal entails inhibiting a natural response to the previously rewarded cue while forming a new association with the previously non-rewarded cue (Day et al 1999, Shettleworth 2010). A large proportion (80%) of *P. sicula* were able to learn the reversal task at the same speed that they learned the initial discrimination task indicating that they had less difficulty inhibiting a previously learned response and greater ease adjusting their behaviour to the changed conditions compared to the other two tested species.

We also found cognitive differences between the invasive *P. sicula* and two non-invasive species, *P. bocagei* and *P. carbonelli*. The two non-invasive species showed a similar pattern of performance and learning in the discrimination and reversal tasks, which was different from that of *P. sicula*. Both non-invasive species needed a similar number of trials to learn the discrimination task, and twice the number of trials to learn the reversal task, which indicates the same relative difficulty to reverse the task between both species (Rajalakshmi and Jeeves 1965). In contrast, *P. sicula* needed more trials to learn the discrimination task, but a comparable number of trials to learn both tasks, suggesting less difficulty in reversing the acquisition (Rajalakshmi and Jeeves 1965). This highlights the benefits of making comparisons with closely related non-invasive species and to use more than one species for comparison to uncover learning patterns (Hazlett et al 2002, Rehage and Sih 2004). For example, Hazlett et al (2002) tested the cognitive ability of two pairs of invasive-native crayfish species and established that the two invasive species had the same memory capacity while the native species likewise presented a similar pattern of memory. Although we collected all three species from urbanised areas, the level of urbanisation at each site may be different and this can lead to differences in the species' cognitive ability. For instance, individuals of the invasive common myna (*Sturnus tristis*) that live in more urbanised environments in Australia are better at solving tasks than individuals from less urbanised locations (Sol et al 2011). In the case of *P. sicula*, although this could be disentangled by comparing native and introduced populations of *P. sicula*, it is not possible to identify the source population. Additionally, despite we collected all three species from urbanised areas, the level of urbanisation in each place may be different and this can lead to differences in the species' cognitive ability. For instance, individuals of the common myna (*Sturnus tristis*), a bird largely introduced in Australia, that live in more urbanised environments are better at solving tasks than individuals that inhabit less urbanised locations (Sol et al 2011). Although this could be disentangled by comparing native and introduced populations of *P. sicula*, similarly as happens to other accidental introductions, it is not possible to know the source population. A surprising result was that both native species did better than the invasive species at the discrimination task and that only one of our predictions was met. Directly measuring cognitive abilities in invasive species has proven to be challenging, and results often do not entirely support all initial predictions (Griffin et al 2013, Bezzina

et al 2014, Chung et al 2017), which seem to reflect the intricate nature of the role of cognition, and behaviour flexibility, in successful invasions.

Behavioural flexibility might not promote invasions as a unique trait, but instead also be correlated with other behavioural traits, which combined might increase the probability of invasion success (Sih et al 2012). For example, it can be an association between behavioural flexibility and social learning the key combination to predict successful invasions (Terkel 1995, Wright et al 2010). This can lead to the quick spread of innovative behaviours through a population, that are advantageous in novel locations (Terkel 1995). Exploratory behaviours correlated with flexibility can also help explain differences in species' invasive potential (Sol et al 2002). It can make invaders to have better foraging efficiency (Rehage and Sih 2004), or to be better at finding suitable shelters in a new environment (Sih et al 2012). Different studies could be incorporated for a better understanding of the potential of cognition in invasions. To further disentangle the differences between species, studies on multiple series of reversal learning (Rajalakshmi and Jeeves 1965, Cauchoix et al 2017) or attentional set-shifting (Szabo et al 2018), or even on tasks that involve complex problem-solving (Chow et al 2018) could provide a better understanding of behavioural flexibility in *P. sicula* or other species.

In summary, of the three species, the invasive *P. sicula* performed the best at reversing a previously learnt association. This difference in behaviour between the invasive and native species might suggest a potential role for behavioural flexibility in *P. sicula* invasion process, as found in other invasive taxa (Sol et al 2002, 2008b, Amiel et al 2011). Interestingly, the non-invasive species presented a similar pattern of learning that was different to that of *P. sicula*. This suggests there may be some constraints to cognition that result in potential trade-offs in learning ability. Cognitive ability is increasingly being recognised for the potentially important role it may play in fitness (Cole et al 2012, Thornton et al 2014). By extension, an animal's cognitive ability could be key to its success as an invader and we need more focused studies addressing this hypothesis. Finally, our study highlights the value of comparing closely related invasive and non-invasive species in order to unravel the behavioural mechanisms linked to the establishment of invasive species.

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ELECTRONIC SUPPLEMENTARY MATERIAL

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RESULTS

Tables

Table S1. Outcome of the statistical models testing if species (*P. sicula*: PS, *P. bocagei*: PB, or *P. carbonelli*: PC) and sex (males: M, females: F) had an effect in the studied variables (a-e), for each task – motor, discrimination, and reversal. Reference levels for all fixed factors are shown in parentheses. N_{ind} represents the number of individuals tested, with N_{obs} for the number of total observations in the dataset. None of the tested interactions were significant, and therefore not included in these final models. For all models, all significant results are indicated in bold.

| | MOTOR TASK | | | | DISCRIMINATION TASK | | | | REVERSAL TASK | | | | |
|-------------------|---|---------|---------|--------|---|---------|---------|--------|--|---------|---------|--------|--------|
| Fixed Effects | a) Number of trials to reach criterion | | | | | | | | | | | | |
| | N _{ind} =60, N _{obs} =60 | | | | N _{ind} =60, N _{obs} =60 | | | | N _{ind} =39, N _{obs} =39 | | | | |
| | β | SE | z-value | P | β | SE | z-value | P | β | SE | z-value | P | |
| | Intercept (PB, F) | 2.107 | 0.091 | 23.219 | <.0001 | 2.479 | 0.126 | 19.708 | <.0001 | 3.163 | 0.095 | 33.370 | <.0001 |
| | Species (PC) | 0.261 | 0.107 | 2.438 | 0.015 | -0.018 | 0.155 | -0.114 | 0.910 | -0.011 | 0.124 | -0.093 | 0.926 |
| | Species (PS) | -0.040 | 0.115 | -0.345 | 0.730 | 0.449 | 0.150 | 2.993 | 0.003 | -0.153 | 0.108 | -1.412 | 0.158 |
| | Sex (Male) | -0.136 | 0.089 | -1.516 | 0.129 | -0.029 | 0.124 | -0.238 | 0.812 | 0.083 | 0.095 | 0.877 | 0.381 |
| | b) Proportion of correct choices | | | | | | | | | | | | |
| | N _{ind} =60, N _{obs} =60 | | | | N _{ind} =60, N _{obs} =60 | | | | N _{ind} =60, N _{obs} =60 | | | | |
| | β | SE | z-value | P | β | SE | z-value | P | β | SE | z-value | P | |
| Intercept (PB, F) | -0.112 | 0.131 | -0.849 | 0.396 | -0.227 | 0.113 | -2.010 | 0.045 | -0.777 | 0.085 | -9.148 | <.0001 | |
| Species (PC) | -0.136 | 0.157 | -0.868 | 0.385 | -0.028 | 0.140 | -0.196 | 0.844 | 0.012 | 0.101 | 0.121 | 0.904 | |
| Species (PS) | 0.024 | 0.165 | 0.146 | 0.884 | -0.140 | 0.128 | -1.093 | 0.274 | 0.164 | 0.105 | 1.557 | 0.119 | |
| Sex (Male) | 0.080 | 0.130 | 0.611 | 0.541 | -0.022 | 0.107 | -0.211 | 0.833 | 0.018 | 0.084 | 0.216 | 0.829 | |
| Fixed Effects | c) Probability of making a correct choice | | | | | | | | | | | | |
| | N _{ind} =60, N _{obs} =502 | | | | N _{ind} =60, N _{obs} =835 | | | | N _{ind} =60, N _{obs} =1745 | | | | |
| | β | SE | z-value | P | β | SE | z-value | P | β | SE | z-value | P | |
| | Intercept (PB, F) | 0.860 | 0.587 | 1.467 | 0.142 | 1.238 | 0.200 | 6.200 | <.0001 | -0.939 | 0.173 | -5.418 | <.0001 |
| | Trials | 0.724 | 0.238 | 3.050 | 0.002 | 0.015 | 0.011 | 1.419 | 0.156 | 0.057 | 0.009 | 6.319 | <.0001 |
| | Species (PC) | -2.022 | 0.589 | -3.435 | 0.001 | -0.124 | 0.223 | -0.558 | 0.577 | 0.095 | 0.181 | 0.525 | 0.599 |
| | Species (PS) | 0.126 | 0.706 | 0.179 | 0.858 | -0.600 | 0.200 | -2.993 | 0.003 | 0.533 | 0.181 | 2.945 | 0.003 |
| | Sex (Male) | 1.229 | 0.497 | 2.472 | 0.013 | -0.087 | 0.159 | -0.548 | 0.584 | -0.037 | 0.145 | -0.256 | 0.798 |
| | d) Latency during trials | | | | | | | | | | | | |
| | N _{ind} =60, N _{obs} =502 | | | | N _{ind} =60, N _{obs} =835 | | | | N _{ind} =60, N _{obs} =1745 | | | | |
| β | SE | z-value | P | β | SE | z-value | P | β | SE | z-value | P | | |
| Intercept (PB, F) | 695.877 | 92.793 | 7.499 | <.0001 | 455.886 | 52.895 | 8.619 | <.0001 | 531.827 | 42.864 | 12.407 | <.0001 | |
| Trials | -35.030 | 13.739 | -2.550 | 0.011 | -6.104 | 4.497 | -1.357 | 0.175 | -8.943 | 2.285 | -3.913 | <.0001 | |

| | | | | | | | | | | | | |
|--|---------------------------------|--------|-------|-------|---|---------------|--------------|------------------|----------------|---------------|--------------|------------------|
| Species (PC) | 182.280 | 99.507 | 1.832 | 0.067 | 242.339 | 58.486 | 4.144 | <.0001 | 189.124 | 49.115 | 3.851 | <.0001 |
| Species (PS) | 134.482 | 95.366 | 1.410 | 0.159 | 94.448 | 56.711 | 1.665 | 0.096 | 68.145 | 50.063 | 1.361 | 0.174 |
| Sex (Male) | 55.453 | 79.818 | 0.695 | 0.487 | 10.185 | 46.681 | 0.218 | 0.827 | 26.716 | 40.444 | 0.661 | 0.509 |
| e) Number of lizards that learnt the task | | | | | | | | | | | | |
| | | | | | N _{ind} =39 N _{obs} =39 | | | | | | | |
| Fixed Effects | | | | | | | | | β | SE | z-value | P |
| Intercept (PB, F) | | | | | | | | | 0.768 | 0.561 | 1.369 | 0.171 |
| Species (PC) | All 60 animals learned the task | | | | All 60 animals learned the task | | | | -1.050 | 0.664 | -1.581 | 0.114 |
| Species (PS) | | | | | | | | | 0.540 | 0.743 | 0.727 | 0.467 |
| Sex (Male) | | | | | | | | | 0.162 | 0.570 | 0.284 | 0.776 |

Table S2. Results of the pairwise comparisons for each task – motor, discrimination, and reversal. N_{ind} represents the number of individuals tested, with N_{obs} being the number of total observations in the dataset. For all pairwise comparisons, all significant results are indicated in bold.

| | MOTOR TASK | | | | DISCRIMINATION TASK | | | | REVERSAL TASK | | | | |
|----------|---|----------|---------|-------------------|---|----------|---------|-------------------|--|----------|---------|-------------------|--------|
| Contrast | a) Number of trials to learn the task | | | | | | | | | | | | |
| | N _{ind} =60, N _{obs} =60 | | | | N _{ind} =60, N _{obs} =60 | | | | N _{ind} =39, N _{obs} =39 | | | | |
| | β | SE | z-value | P _{corr} | β | SE | z-value | P _{corr} | β | SE | z-value | P _{corr} | |
| | P. bocagei - P. carbonelli | -0.261 | 0.107 | -2.438 | 0.039 | 0.018 | 0.155 | 0.114 | 0.993 | 0.011 | 0.123 | 0.093 | 0.995 |
| | P. bocagei - P. sicula | 0.040 | 0.115 | 0.345 | 0.936 | -0.449 | 0.150 | -2.993 | 0.008 | 0.153 | 0.108 | 1.412 | 0.335 |
| | P. carbonelli - P. sicula | 0.301 | 0.108 | 2.777 | 0.015 | -0.467 | 0.150 | -3.105 | 0.005 | 0.142 | 0.123 | 1.153 | 0.481 |
| Contrast | b) Proportion of correct choices | | | | | | | | | | | | |
| | N _{ind} =60, N _{obs} =60 | | | | N _{ind} =60, N _{obs} =60 | | | | N _{ind} =60, N _{obs} =60 | | | | |
| | β | SE | z-value | P _{corr} | β | SE | z-value | P _{corr} | β | SE | z-value | P _{corr} | |
| | P. bocagei - P. carbonelli | 0.136 | 0.157 | 0.868 | 0.661 | 0.028 | 0.140 | 0.196 | 0.979 | -0.012 | 0.101 | -0.121 | 0.992 |
| | P. bocagei - P. sicula | -0.024 | 0.165 | -0.146 | 0.988 | 0.140 | 0.128 | 1.093 | 0.518 | -0.164 | 0.105 | -1.557 | 0.265 |
| | P. carbonelli - P. sicula | -0.160 | 0.158 | -1.014 | 0.568 | 0.113 | 0.129 | 0.871 | 0.659 | -0.152 | 0.102 | -1.482 | 0.300 |
| Contrast | c) Probability of making a correct choice | | | | | | | | | | | | |
| | N _{ind} =60, N _{obs} =502 | | | | N _{ind} =60, N _{obs} =835 | | | | N _{ind} =60, N _{obs} =1745 | | | | |
| | β | SE | z-value | P _{corr} | β | SE | z-value | P _{corr} | β | SE | z-value | P _{corr} | |
| | P. bocagei - P. carbonelli | 2.022 | 0.589 | 3.435 | 0.002 | 0.124 | 0.223 | 0.558 | 0.842 | -0.095 | 0.181 | -0.525 | 0.859 |
| | P. bocagei - P. sicula | -0.126 | 0.706 | -0.179 | 0.983 | 0.600 | 0.200 | 2.993 | 0.008 | -0.533 | 0.181 | -2.945 | 0.009 |
| | P. carbonelli - P. sicula | -2.149 | 0.621 | -3.458 | 0.002 | 0.475 | 0.197 | 2.415 | 0.042 | -0.438 | 0.177 | -2.474 | 0.036 |
| Contrast | d) Latency during trials | | | | | | | | | | | | |
| | N _{ind} =60, N _{obs} =502 | | | | N _{ind} =60, N _{obs} =835 | | | | N _{ind} =60, N _{obs} =1745 | | | | |
| | β | SE | z-value | P _{corr} | β | SE | z-value | P _{corr} | β | SE | z-value | P _{corr} | |
| | P. bocagei - P. carbonelli | -182.280 | 99.507 | -1.832 | 0.159 | -242.339 | 58.486 | -4.144 | <.0001 | -189.124 | 49.115 | -3.851 | <.0001 |
| | P. bocagei - P. sicula | -134.482 | 95.366 | -1.410 | 0.336 | -94.448 | 56.711 | -1.665 | 0.219 | -68.145 | 50.063 | -1.361 | 0.362 |
| | P. carbonelli - P. sicula | 47.799 | 98.949 | 0.483 | 0.879 | 147.891 | 56.630 | 2.612 | 0.025 | 120.979 | 49.512 | 2.443 | 0.039 |
| Contrast | e) Number of lizards that learnt the task | | | | | | | | | | | | |
| | | | | | | | | | N _{ind} =39, N _{obs} =39 | | | | |
| | | | | | | | | | β | SE | z-value | P _{corr} | |
| | All 60 animals learned the task | | | | All 60 animals learned the task | | | | 1.050 | 0.664 | 1.581 | 0.254 | |
| | | | | | | | | | -0.540 | 0.742 | -0.727 | 0.748 | |
| | | | | | | | | | -1.589 | 0.718 | -2.214 | 0.069 | |

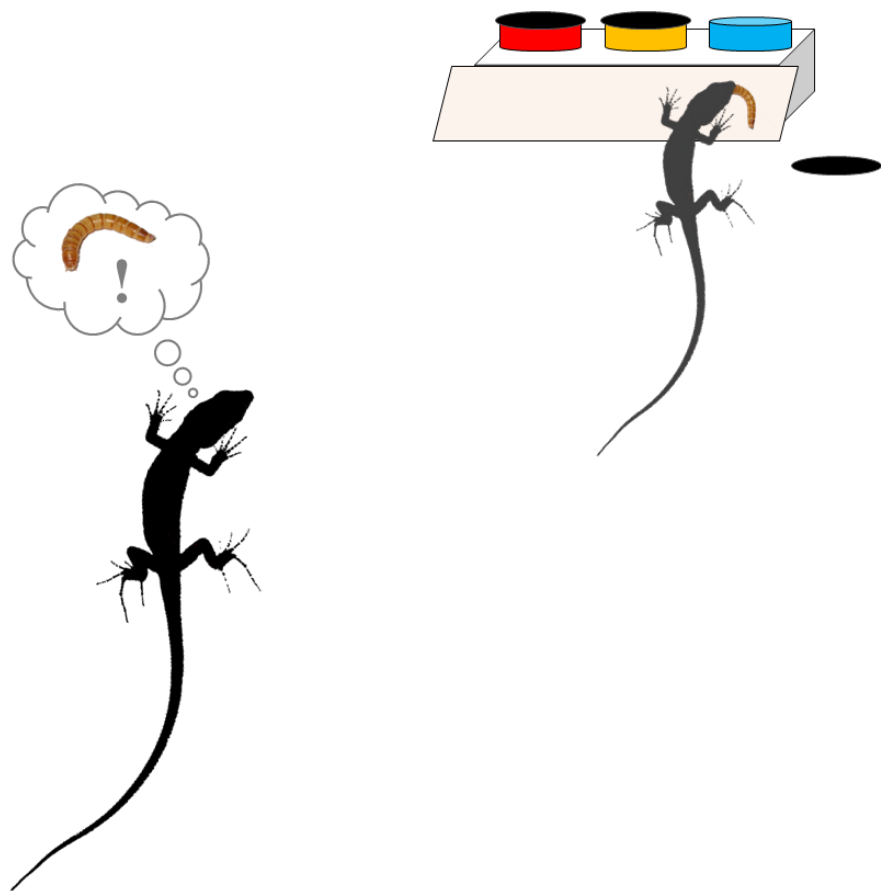
Chapter II

Learning from others: An invasive lizard uses social information from both conspecifics and heterospecifics

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Learning from others: An invasive lizard uses social information from both conspecifics and heterospecifics

ABSTRACT

Species that are able to solve novel problems through social learning from either a conspecific or a heterospecific may gain a significant advantage in new environments. We tested the ability of a highly successful invasive species, the Italian wall lizard *Podarcis sicula*, to solve a novel foraging task when social information was available from both a conspecific and an unfamiliar heterospecific (*Podarcis bocagei*). We found that Italian wall lizards that had access to social information made fewer errors, regardless of whether the demonstrator was a conspecific or a heterospecific, compared to Italian wall lizards that individually learnt the same task. We suggest that social learning could be a previously underappreciated, advantageous mechanism facilitating invasions.

KEYWORDS

Podarcis sicula; biological invasions; social learning; heterospecific learning; cognition

INTRODUCTION

Invasive species are a global problem with severe economic and environmental impacts. Despite all the attention, the mechanisms underlying successful invasions are often unclear, although behaviour and cognition are thought to play a key role [1,2]. The ability to learn from conspecifics (social learning) may give individuals an advantage in novel environments [3]. For example, black rats (*Rattus rattus*) were able to invade a new patch of pine forest after innovating a feeding method that spread to the rest of the population through social learning [3]. Social learning thus has the potential to influence a species' invasive success, although this is rarely considered. Animals can use cues provided by conspecifics to minimize risk and make a wide range of decisions that may impact fitness [4]. We propose that the same can be true when learning from a different species. It is likely that heterospecific learning is more common than previously believed [5] because so many species make use of heterospecific cues to make decisions about escaping predators [6], where to find suitable habitat [7], or food sources [8]. During an introduction event, where conspecifics may be present in low numbers and unfamiliar with their new environment [4], the ability to learn from a different species could represent a powerful shortcut to individual learning.

To the best of our knowledge, the use of heterospecific learning by an invasive species has never previously been tested. To investigate this, we used the invasive Italian wall lizard *Podarcis sicula*, which has established populations in several countries outside its native range and that commonly interacts with native lizards, through competition or hybridization [9]. Many introductions of the Italian wall lizard occur because individuals have been accidentally stowed away in human cargo and transported long distances from their native range [9]. A robust test of heterospecific learning in the context of an invasion is to select a species that they have never encountered before. There are 23 species of wall lizards spread across the Mediterranean Basin, which means it is highly likely that in any invasion an Italian wall lizard will encounter another *Podarcis*. We tested the hypothesis that Italian wall lizards use social information to learn a novel foraging task. We predicted that they will solve a foraging task more rapidly when social information is available from either a conspecific or heterospecific, compared to a control in which social information is absent.

MATERIAL AND METHODS

Treatments and social learning task

We collected 43 female *Podarcis sicula* from Lisbon and 10 *Podarcis bocagei* from Vairão, Portugal (details in electronic supplementary material). We randomly allocated lizards to two different treatments: social and individual (control) learners. In each learning treatment, the focal species *P. sicula* was paired with either a conspecific or heterospecific (*P. bocagei*; table 1). Each pair shared an opaque enclosure (320 mm W × 480 mm L × 300 mm H) that was divided by both a fixed transparent barrier (Plexiglas) and a removable opaque (wood) barrier. Each lizard occupied one side of the enclosure in a protocol similar to [10,11].

Lizards had to solve a discrimination task in which they had a choice of three dishes with different coloured (blue, yellow and red) removable lids. Only one dish (blue) contained an accessible food reward (a live mealworm). The dishes were placed on an elevated platform (4 cm tall) with a ramp that provided easy access (figure 1a). The location of the colours was randomized between pairs and trials, but the observers in the social treatment always had the same arrangement of dishes as the respective demonstrator. All demonstrators were trained to remove the blue lid to receive a reward before experiments began. Demonstrators were only able to remove the blue lid (yellow and red lids were fixed) to ensure that the observer only received reliable information during trials. At the same time, all experimental lizards were trained to eat from a dish.

Table 1. The number of animals in each treatment and with each demonstrator species (N), the latency to the correct choice (s), the number that reached learning criterion (N_{learners}), and the number that were successful in the first trial ($N_{\text{first trial}}$). Standard error (SE) follows all means.

| Observer | Treatment | Demonstrator | N | Proportion of correct choices | Latency | N_{learners} | Number of trials to reach learning criterion | $N_{\text{first trial}}$ |
|------------------|------------|-------------------|----|-------------------------------|----------|-----------------------|--|--------------------------|
| <i>P. sicula</i> | Social | <i>P. sicula</i> | 10 | 0.48 ± 0.04 | 348 ± 62 | 4 | 26.00 ± 5.37 | 4 |
| | | | 21 | 0.46 ± 0.03 | 384 ± 44 | 8 | 24.13 ± 2.98 | 6 (28.6%) |
| <i>P. sicula</i> | Social | <i>P. bocagei</i> | 11 | 0.44 ± 0.03 | 416 ± 63 | 4 | 22.25 ± 3.22 | 2 |
| <i>P. sicula</i> | Individual | <i>P. sicula</i> | 8 | 0.35 ± 0.04 | 583 ± 96 | 1 | 25.00 ± NA | 1 |
| | | | 16 | 0.34 ± 0.02 | 400 ± 67 | 2 | 30.00 ± 5.00 | 4 (25.0%) |
| <i>P. sicula</i> | Individual | <i>P. bocagei</i> | 8 | 0.33 ± 0.02 | 218 ± 27 | 1 | 35.00 ± NA | 3 |

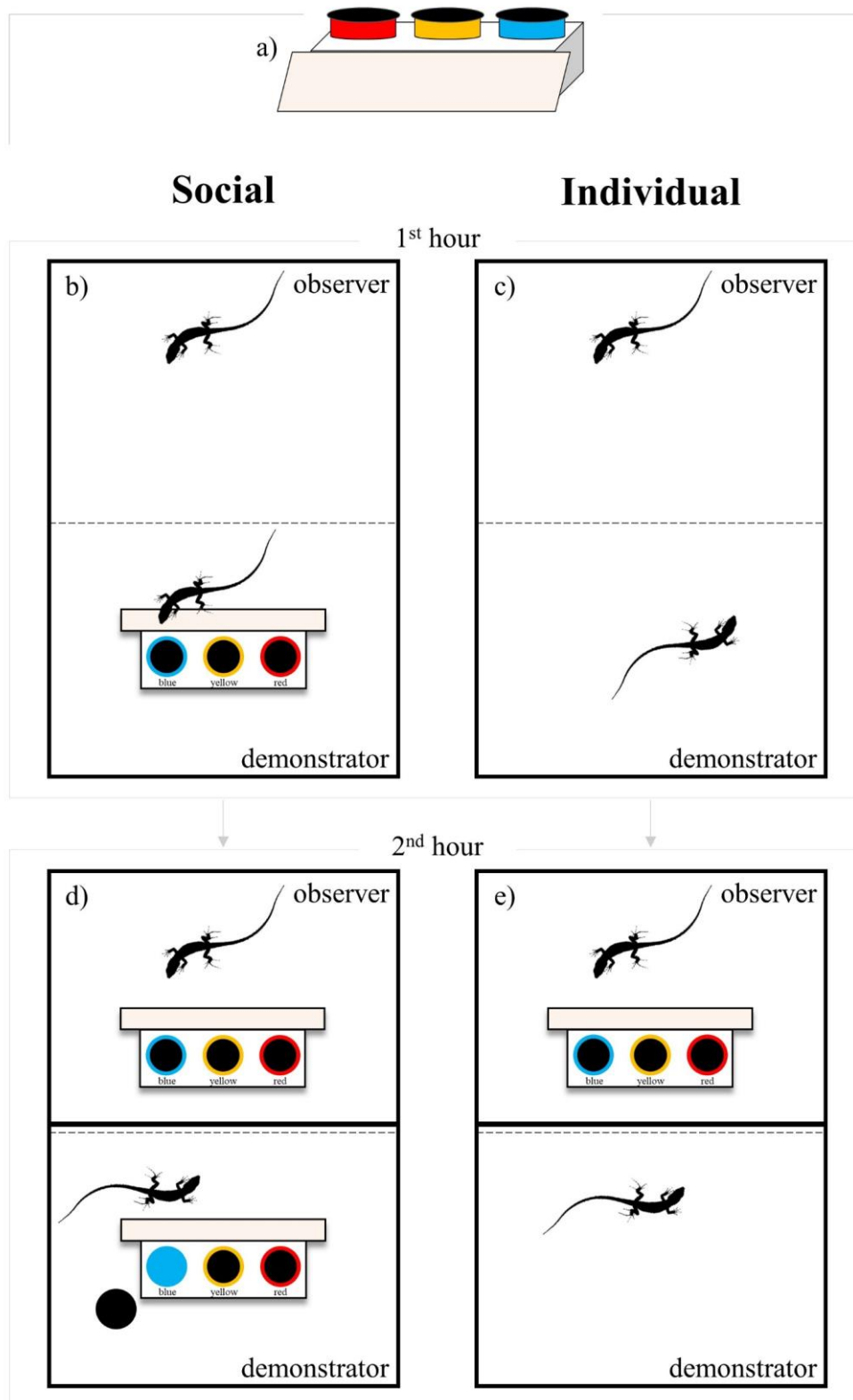


Figure 1. The experimental apparatus (a) and protocol. The social treatment (b) observed a demonstrator performing the discrimination task for 1 h, while the individual treatment (c) observed another lizard in the absence of the apparatus. After the opaque barrier was reinserted all observers were presented with the task for another hour (d,e).

Experimental set up

Each trial began by removing the opaque barrier between a pair, leaving only the transparent barrier. For the social treatment, the apparatus was placed in the demonstrator's area with the ramp facing the observers (figure 1b). For the individual treatment (control), the observer was able to view another lizard in the absence of a task (figure 1c). Details in the electronic supplementary material.

After 1 h, the opaque barrier between the pair was reinserted and the apparatus was placed in the observer's area, mirroring the placement in the demonstrator's area (figure 1d,e). Observers performed the task correctly if they opened the blue lid first and ate the mealworm. If lizards did not make a choice, the trial was not counted. If a demonstrator did not perform the task, we halted the trial and did not give the task to the focal lizard. We gave the lizards 40 trials to reach our learning criterion and they were considered to have learnt the task once they made 7/7 or 7/8 correct choices [11]. Although we did not test for robustness, this learning criterion is significant according to a binomial probability, which is conservative for this experiment because the task consisted of three choices. All trials were remotely video recorded with CCTV cameras and scored by I.D.M. From each video, we recorded whether the lizard performed the task correctly (1, opened the blue dish) or incorrectly (0, opened the red or yellow dishes), and the latency (s) from the moment the apparatus was available until the correct dish was opened.

Statistical analyses

Statistical analyses were performed in R version 3.4.2 [12], to examine if there were differences between learning treatments (social or individual) and demonstrator species (*P. sicula* or *P. bocagei*) in:

- a) the number of lizards that learnt the task (learnt = 1, not learnt = 0) using a generalized linear model (GLM) with a binomial distribution (by using the function *glm* from the R package *stats* [13]);
- b) the number of trials until learning criterion using a GLM with a Poisson distribution;

- c) the proportion of correct choices (the number of correct choices over the number of total trials each lizard performed) using a GLM with a binomial distribution;
- d) the probability of making a correct choice within a trial (correct = 1 and incorrect = 0) using a generalized linear mixed effect model (GLMM) with a binomial distribution (using the function *glmer* from the *lme4* R package [14]). Trial number was included as an additional predictor variable in this model. We also included a random intercept and slope for lizard identity across trials to account for dependency among repeated observations of the same individual;
- e) latency until the correct choice within a trial using a linear mixed effect model (LMM; Gaussian distribution), with the function *lmer* from the *lme4* package [14]. The LMM contained the same variables, fixed and random, as the GLMM above (d). Details in the electronic supplementary material.

RESULTS

In the social treatment 38% (8/21) of lizards met the learning criterion, while in the individual treatment 13% (2/16) of lizards reached the learning criterion (Table 1). Neither the number of lizards that learnt between treatments, nor demonstrator species, were significantly different (Table 2a). Similarly, the treatment or demonstrator species did not affect the number of trials needed to reach the learning criterion (Table 2b). The social treatment had a significantly higher proportion of correct choices, but no effect regarding which species was demonstrating the task (Table 2c, Figure 2a). The probability of making the correct choice within a trial was significantly higher in the social treatment, while this did not differ between demonstrator species (Table 2d, Figure 2b). Latency to make a correct choice within a trial (s) was affected by an interaction between demonstrator species and treatment (Table 2e); *P. sicula* (social treatment) that were observing *P. bocagei* took significantly longer to correctly complete the trials ($\beta = -209.440 \pm 61.601$, $z\text{-value} = -3.400$, $P = 0.004$). All other treatment and species interaction comparisons were non-significant (electronic supplementary material, Table S1).

Table 2. Outcomes of statistical models. Number of individuals (N_{ind}) and of observations (N_{obs}) are indicated; significant values are in bold.

| a) Number of lizards that learnt | | | | |
|---|-----------------|---------------|----------------|-------------------|
| $N_{\text{ind}} = 37$ | | | | |
| | β | <i>SE</i> | <i>z-value</i> | <i>P</i> |
| Intercept (Individual - <i>P. bocagei</i>) | -2.004 | 0.857 | -2.339 | 0.019 |
| Treatment (Social) | 1.464 | 0.880 | 1.663 | 0.096 |
| Demonstrator (<i>P. sicula</i>) | 0.114 | 0.773 | 0.147 | 0.883 |
| b) Number of trials taken to reach criterion | | | | |
| $N_{\text{ind}} = 10$ | | | | |
| | β | <i>SE</i> | <i>z-value</i> | <i>P</i> |
| Intercept (Individual - <i>P. bocagei</i>) | 3.379 | 0.224 | 15.083 | < 0.001 |
| Treatment (Social) | -0.222 | 0.229 | -0.967 | 0.333 |
| Demonstrator (<i>P. sicula</i>) | 0.050 | 0.188 | 0.267 | 0.789 |
| c) Proportion of correct choices | | | | |
| $N_{\text{ind}} = 37$ | | | | |
| | β | <i>SE</i> | <i>z-value</i> | <i>P</i> |
| Intercept (Individual - <i>P. bocagei</i>) | -1.119 | 0.095 | -11.729 | < 0.001 |
| Treatment (Social) | 0.240 | 0.105 | 2.287 | 0.022 |
| Demonstrator (<i>P. sicula</i>) | 0.073 | 0.104 | 0.703 | 0.482 |
| d) Probability of learning within a trial | | | | |
| $N_{\text{ind}} = 37, N_{\text{obs}} = 1333$ | | | | |
| | β | <i>SE</i> | <i>z-value</i> | <i>P</i> |
| Intercept (individual - <i>P. bocagei</i>) | -0.974 | 0.164 | -5.950 | <0.001 |
| Trial number | 0.011 | 0.006 | 1.900 | 0.057 |
| Treatment (Social) | 0.481 | 0.145 | 3.322 | 0.001 |
| Demonstrator (<i>P. sicula</i>) | 0.126 | 0.134 | 0.943 | 0.345 |
| e) Latency until correct choice within a trial | | | | |
| $N_{\text{ind}} = 37, N_{\text{obs}} = 1333$ | | | | |
| | β | <i>SE</i> | <i>z-value</i> | <i>P</i> |
| Intercept (Individual - <i>P. bocagei</i>) | 382.199 | 45.096 | 8.475 | < 0.001 |
| Trial number | -6.392 | 1.910 | -3.347 | 0.001 |
| Treatment (Social) | 141.742 | 57.529 | 2.464 | 0.014 |
| Demonstrator (<i>P. sicula</i>) | 209.440 | 61.601 | 3.400 | 0.001 |
| Treatment:Demonstrator (Social: <i>P. sicula</i>) | -222.267 | 82.356 | -2.699 | 0.007 |

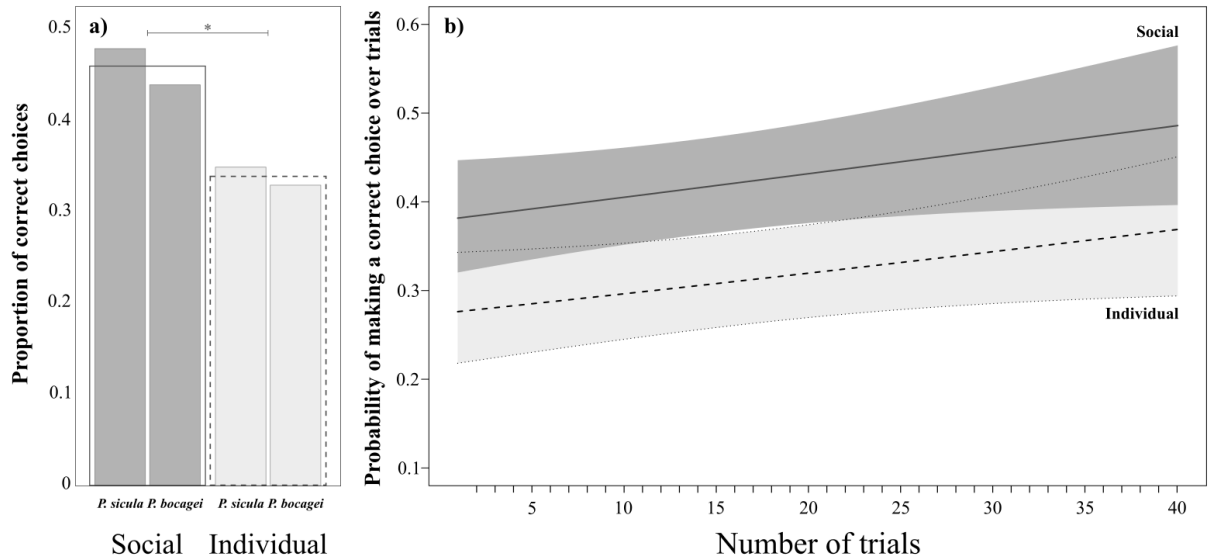


Figure 2. The proportion of correct choices during the task (a), and the probability of making a correct choice across 40 trials (b). Social treatment is represented in dark grey (solid lines), and the individual treatment in light grey (dashed lines). In (a) the outlined bars show treatment average of raw data, whereas the shaded bars are demonstrator-specific treatment averages. In (b) we plotted data predicted from models; shaded polygons on either side of the fitted lines are 95% confidence intervals.

DISCUSSION

We show that the invasive Italian wall lizard is able to use social information to solve a novel foraging task. While the proportion of individuals that reached the learning criterion was relatively low, lizards in the social treatment made fewer errors and had a higher probability of making a correct choice within a trial. Notably, Italian wall lizards used social information from both conspecifics and heterospecifics. However, the number of lizards that learnt, and the number of trials taken to learn the task, were not significantly different between social and individual learning treatments. While there is a relatively rich literature on how animals use heterospecific cues to make decisions on where to forage [8] or when to seek refuge [6], the idea that animals learn from other species has received little attention [5,15]. Overall, our results add to the accumulating evidence that non-avian reptiles can learn from conspecifics (e.g. [10,16,17]) and we report the first instance of heterospecific learning in an invasive species.

Biological invasions can be complex and dynamic. It may be over-simplistic to simply focus on an invasive species' traits and abilities, without considering the community into which an organism is introduced. If for example, their new environment contains closely related native species, they may make use of subtle behaviours to obtain important information about the location of food and resources. This information can form the basis for later social learning.

Our results have important implications for the field of invasion biology because they not only support previous findings that cognitive ability can play an important role in determining the success of an invasion [2], but that social learning may be an additional mechanism facilitating the establishment of invasive species in novel environments. During the course of an invasion, invasive species interact with a host of species that include conspecifics, native species, predators and prey [7,18]. Some of these organisms are in competition for resources. By using social information from both conspecifics and heterospecifics, invasive species may gain a small but significant advantage needed for success.

ETHICS: Research approved by the Macquarie University Animal Ethics Committee (ARA2015/038) and by the Portuguese Institute for Conservation of Nature and Forests (ICNF) (License 695/2016/CAPT and 157/2017/CAPT).

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ELECTRONIC SUPPLEMENTARY MATERIAL

Damas-Moreira I, Oliveira D, Santos JL, Riley JL, Harris DJ, Whiting MJ. 2018 Learning from others: an invasive lizard uses social information from both conspecifics and heterospecifics. *Biol. Lett.*

Some information may be repeated from the manuscript to make the supplementary material easier to follow.

Study species

The invasive Italian wall lizard (*Podarcis sicula*) is a small lacertid native to the Italian Peninsula and the Adriatic coast. Introduced Italian wall lizards often thrive in locations where there is a native *Podarcis* species or at least another small lacertid (e.g. on Mediterranean islands: *P. lilfordi*, *P. raffonei* or *P. melisellensis*; in Lisbon: *P. virescens*; or, in Athens: *Chalcides ocellatus*), and can interact with them, for example, by competing or hybridizing [1].

Furthermore, Italian wall lizards frequently become established following long-range transportation. As such, Italian wall lizards represent a good model for testing heterospecific social learning in an invasive species. The population of wall lizards (*P. sicula*) we sampled was accidentally introduced to Lisbon in 1998, likely with plant ornaments brought from Tuscany, Italy, for the world exhibition that took place that year [2,3]. Bocage's lizard (*P. bocagei*) has a restricted range in the north-western Iberian Peninsula, so these two species have not been in contact before, and the closest populations are separated by > 300 km.

Collection and husbandry

We used only females to avoid the potential confounding effects of male social feedback, as male *Podarcis* aggressively compete for space. We collected all lizards from the wild, and all had complete tails. *Podarcis sicula* were collected in Lisbon (western Portugal, N 38° 45' 43.8"; W 9° 5' 41.7") from a population introduced in 1998 [1]. *Podarcis bocagei* were collected in Vairão (northwestern Portugal, N 41° 18' 37.6", W 8° 40' 32.1"). After collection from the wild, lizards were transported to CIBIO-InBIO facilities at the University of Porto on the same day. While in the lab, lizards had access

to water and a refuge, and were kept under a light:dark 12:12 h photoperiod in a room maintained at a constant temperature of 26°C during the day [4].

Experimental pairs

We paired all lizards according to their body size (snout-vent length, SVL) similarity (each lizard paired with the next closest lizard in size). On average, *P. sicula* paired with *P. sicula* had a mean difference (\pm standard error, SE) of 2.7 ± 1.0 mm, and *P. sicula* paired with *P. bocagei* had a mean difference of 7.6 ± 1.1 mm. The greater difference in SVL between species is because *P. sicula* are naturally larger than *P. bocagei*. Regardless of the slightly greater difference in SVL among pairs of different species, 81.1% of the animals were less than 10 mm different in SVL and body size differences did not preclude social learning. During our experiment, lizard pairs were never in physical contact. Each lizard occupied one side of the test enclosure in a protocol similar to [5,6].

The learning task

Lizards had to remove a lid from a well in order to access a food reward. We selected a foraging task as this is a crucial task for an animal's survival in a novel environment. Furthermore, food promotes animal motivation during experiments and relies on positive reinforcement (compared with a refuge task for example). The diameter of each lid covering a food well was 45 mm and each dish was 35 mm, which allowed 5 mm of the lid to extend around the dish in order to facilitate its removal. All dishes had one mealworm inside, thereby controlling for auditory and chemical cues. In the case of the yellow and red dishes, the mealworm was covered by mesh and inaccessible to the lizard [6]. In the blue dish, the mealworm was accessible.

Training and habituation

Before experiments, all lizards were fed with one mealworm twice a day during weekdays, for about 20 days. Demonstrators were trained on the task, so they were fed using the apparatus, to allow habituation. During this training period, we included one mealworm inside the blue dish, and glued the yellow and

red lids to the dishes to make it impossible for the lizard to open. Besides the demonstrators, all the lizards were habituated to eating out of a transparent dish. The goal was to get the lizards to associate the dish with the presence of food inside.

Experimental set-up

All All lizards had constant access to a refuge and water. At the beginning of each experimental trial, the focal lizard's refuge and water bowls were removed and replaced at the end of each trial. We removed these items to ensure the focal individuals were not hiding and had an unobstructed view of the demonstrator during the trial.

Lizards were given 2 trials per day for 5 days, followed by a two-day break [6]. After each trial, all lids and dishes from the experimental lizards (but not demonstrators) were cleaned with disinfectant wipes and redistributed among all the lizards in the lab. The apparatuses, upon which the dishes were placed, were not cleaned but were always the same for each lizard.

If after 40 trials lizards did not meet our learning criterion, they were considered not to have learnt the task.

Statistical analyses

Before all statistical analyses, we used the protocol from [7] to explore our data and ensure it fitted model assumptions, such as having the appropriate data integrity, no influential outliers, or no collinearity between predictor variables within a model.

Following the order of the models described in the manuscript, we analysed the following interactions: for models a), b), and c) we tested an interaction between learning treatment and demonstrator species, which was removed from the final models because they were not significant; for model d) we analysed an interaction between trial number and learning treatment that was later removed because it was not significant; and for model e) we included an interaction between demonstrator species and trial number, and between demonstrator species and learning treatment. We removed the interaction demonstrator

species \times trial number from the final model because it was not significant, but maintained the interaction demonstrator species \times learning treatment in the final model because it was significant.

We ensured all models fitted their assumptions after they were run, as appropriate, depending on the model's distribution, homogeneity of variance, normality of residuals, and lack of overdispersion. For all models, α was set at 0.05. For multiple comparisons, when interactions were significant, we used the function *lsmeans* from the *lsmeans* R package and corrected *P*-values using Tukey's HSD multiplicity adjustment [8].

Results

Latency to make a correct choice within a trial (s) was significantly affected by an interaction between demonstrator species and treatment. Pairwise analyses showed that *P. sicula* within the social treatment that were observing *P. bocagei* took significantly longer to correctly complete the task, but all other treatment and species interaction comparisons were non-significant (Table S1).

Tables

Table S1. Pairwise comparisons for a significant interaction effect between demonstrator species and learning treatment on latency to make a correct choice within a trial (s). Significant values are indicated in bold.

| Contrast | β | SE | z-value | P_{corr} |
|---|-----------------|---------------|---------------|--------------|
| Individual, <i>P. bocagei</i> - Social, <i>P. bocagei</i> | -141.742 | 57.529 | -2.464 | 0.066 |
| Individual, <i>P. bocagei</i> - Individual, <i>P. sicula</i> | -209.440 | 61.601 | -3.400 | 0.004 |
| Individual, <i>P. bocagei</i> - Social, <i>P. sicula</i> | -128.915 | 58.865 | -2.190 | 0.126 |
| Social, <i>P. bocagei</i> - Individual, <i>P. sicula</i> | -67.698 | 57.601 | -1.175 | 0.643 |
| Social, <i>P. bocagei</i> - Social, <i>P. sicula</i> | 12.827 | 54.659 | 0.235 | 0.996 |
| Individual, <i>P. sicula</i> - Social, <i>P. sicula</i> | 80.525 | 58.937 | 1.366 | 0.521 |

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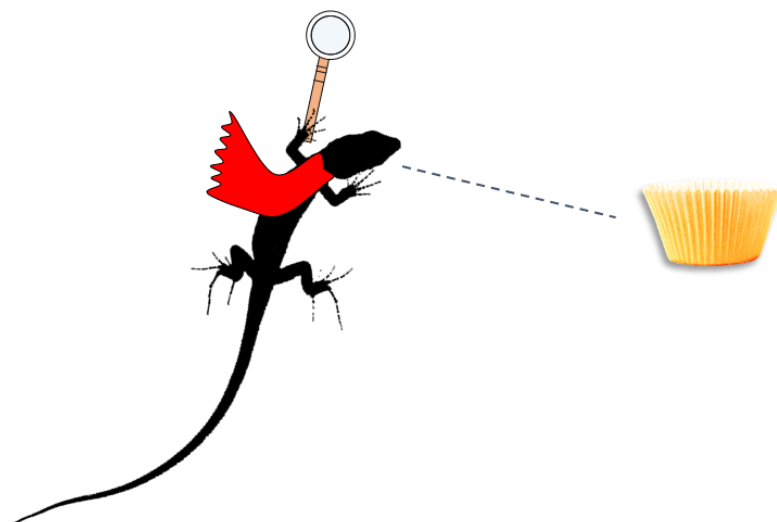
Chapter III

Can behaviour explain invasion success?

A comparison between sympatric invasive and native lizards

Isabel Damas-Moreira, Julia L Riley, D James Harris, Martin J Whiting

Manuscript submitted



Can behaviour explain invasion success?

A comparison between sympatric invasive and native lizards

ABSTRACT

To reduce the impact of biological invasions, we need to understand the behavioural mechanisms that enable some species to be successful invaders. Testing differences in behaviour between sympatric congeneric species with different invasive potentials is an opportunity to study specific behavioural traits associated with invasion success. Using the invasive Italian wall lizard *Podarcis sicula* and a non-invasive congeneric, the green Iberian wall lizard *Podarcis virescens*, which live in sympatry in a location that is novel for *P. sicula*, we tested their exploratory behaviour, neophobia, and boldness – all traits that should promote invasion success. The invasive *P. sicula* was more exploratory, bold, and neophilic than the sympatric native *P. virescens*. Native lizards had highly repeatable behaviour, whereas in *P. sicula* boldness was the only behavioural trait that was repeatable. The behavioural traits of the native species, but not the invasive species, were correlated. A lack of correlation between behavioural traits, as well as a lack of repeatability in the two of the three behavioural traits, suggests greater levels of behavioural plasticity in *P. sicula*, which may also explain the success of this lizard during invasions. Our experiment highlights the potential importance of behavioural traits in invasions, and provides insight into why *P. sicula* is such a successful invader.

KEYWORDS

Podarcis sicula; biological invasions; exploration; boldness; neophobia

INTRODUCTION

Biological invasions have enormous ecological and economic costs, and are of great concern worldwide. In order to prevent or limit the impact of invasions, we need to better understand what makes some species successful, and others unsuccessful, invaders (Carere and Gherardi 2013, Chapple et al. 2012, Holway and Suarez 1999). Recent work has begun focusing on how behavioural traits at the population- and individual-level promote invasion success (Carere and Gherardi 2013, Chapple et al. 2012, Holway and Suarez 1999, Sih et al. 2012, Wolf and Weissing 2012). In general, invasive species have been associated with higher levels of aggression (Downes and Bauwens 2002, Weis 2010), exploration, and boldness (Chapple et al. 2011, Monceau et al. 2015, Short and Petren 2008) than non-invasive species. They may be more likely to disperse (Rehage and Sih 2004) and they may be more efficient at foraging (Pintor and Sih 2009, Short and Petren 2008). These behaviours likely promote the progress and success of a species during different stages of the invasion process (Chapple et al. 2012). For example, high levels of boldness and exploration can determine whether individuals leave their native range, enter a transport vector, and exit in a novel location (Chapple et al. 2011, 2012). Once in a new environment, the establishment of a species is often associated with higher levels of boldness and exploration (Chapple et al. 2012, Monceau et al. 2015, Short and Petren 2008), and lower levels of neophobia (Candler and Bernal 2015, Griffin et al. 2016). These traits could promote the exploitation of resources and also give invasive species an advantage over native species. During establishment, higher aggression and foraging levels can also give invasive species a competitive advantage over native species, which may increase their establishment success (Downes and Bauwens 2002, Weis 2010). After establishment, the expansion of a population's range can depend on the individual's affinity for dispersal, its boldness and exploratory behaviour, aggression levels, and sociability (Cote et al. 2010, Gruber et al. 2017a, Michelangeli et al. 2017, Rehage and Sih 2004).

An individual's personality (i.e. repeatable behaviours across time; Réale et al. 2007, Wolf and Weissing 2012) can be correlated across contexts, forming a 'behavioural syndrome' (Chapple et al. 2012, Sih et al. 2004). If this correlation enhances a species' invasion success it is termed an 'invasion syndrome' (Chapple et al. 2012), and can determine the success of different invaders in distinct ways (Cote et al. 2010, Dame and Petren 2006, Michelangeli et al. 2017, Monceau et al. 2015, Pintor et al.

2008, 2009, Rehage and Sih 2004). For example, the invasion success of the signal crayfish, *Pacifastacus leniusculus*, is because of a positive correlation between aggression and foraging activity (Pintor et al. 2009), while in the invasive mosquitofish, *Gambusia affinis* (Cote et al. 2010) and in the wasp *Vespa velutina* (Monceau et al. 2015), it is due to a positive correlation between their boldness, activity, and exploratory behaviour. Interestingly, the correlation found in invasive *V. velutina* was also found in the native *V. crabro* (with the invasive species exhibiting greater boldness, activity, and exploration), yet the invasive species did not behave consistently while the native species did (Monceau et al. 2015). This suggests that invasive species might be more plastic in their behaviour than native species, which can be a significant advantage when dealing with new challenges and opportunities that arise from novel environments (Sih et al. 2012). The native species likely experience consistent selection pressure on behavioural traits, which promotes repeatability across time (Sih et al. 2012).

Behavioural differences between a successful invader and a congeneric native species can shed light on behaviour that promotes invasion success (Holway and Suarez 1999, Rehage et al. 2005). If both congeneric species with different invasive potential are sympatric, then any environmental effects are minimised (as they live sympatrically), and there is an opportunity to better understand behavioural traits associated with invasion success. This comparison not only gives insight into why particular species are successful invaders, but also helps us understand any potential impacts on native species (Carere and Gherardi 2013, Holway and Suarez 1999, Phillips and Suarez 2012). The Italian wall lizard, *Podarcis sicula*, is an invasive species native to the Italian Peninsula and the Adriatic coast, but is established in several countries outside its native range (CABI 2018, Carretero and Silva-Rocha 2015). *Podarcis sicula* spreads mainly through human transport vectors (Carretero and Silva-Rocha 2015, Kraus 2009, Silva-Rocha et al. 2012, 2014), reaching high-density populations and affecting native lizards in new locations (Capula 1993, 2002, Carretero and Silva-Rocha 2015, Downes and Bauwens 2002, Kraus 2009). About 20 years ago, a population was accidentally introduced to Lisbon, Portugal, from Tuscany, Italy (González de la Vega et al. 2001, Silva-Rocha et al. 2012). This population lives in sympatry with the congeneric green Iberian wall lizard *Podarcis virescens*, but not in syntopy (although they can be found as close as 50 m from each other; Ribeiro 2017). *Podarcis virescens* is a non-invasive lizard that can occur in transport hubs but has never been recorded in an

established population outside its native range. These two congeneric species thus have different invasive potential and live under very similar environmental conditions (i.e., similar predation pressure, urbanisation level, habitat, humidity, temperature, and prey availability) which makes them a model system to study the role behaviour plays in facilitating invasion success.

We quantified three behaviours typically associated with a species' invasive potential – exploration, neophobia, and boldness (Chapple et al. 2012, Griffin et al. 2016, Phillips and Suarez 2012), and tested how they differed between the invasive *P. sicula* and the native *P. virescens*. We predicted that *P. sicula* would be more exploratory, bold, and less neophobic, given its potential to travel to new places, prosper there, and displace native species (CABI 2018). We also investigated the repeatability of the behavioural traits in each species. We expected *P. sicula* individuals to be less repeatable than *P. virescens* individuals in their behavioural traits, indicating more plasticity in the invasive species. Finally, we investigated if behavioural traits were correlated within each species, to explore the existence of 'behavioural' and/or 'invasion' syndromes.

MATERIAL AND METHODS

Study species and captive conditions

We collected 26 male *P. sicula* and 29 *P. virescens* from the wild and assayed their behavioural traits. We did not test females as the reproductive status of wild-caught females (if gravid or not, or if recently post-parturient) cannot be assessed with certainty, and the different hormones acting in each reproductive stage could greatly influence their behaviour. Male *Podarcis* sp. typically copulate from March to July, and testosterone levels tend to be synchronous within a locality and breeding season, reducing this problem in males of our model species (Carretero 2006). However, the invasive potential of the delicate skink *Lampropholis delicata* is similar for both sexes (tested for exploration, boldness, activity, sociability, and foraging activity; Michelangeli et al. 2016).

We collected all lizards (both species) during June (spring) from Parque das Nações, Lisbon, Portugal (N 38° 45' 41.7, W 9° 5' 43.8") on two different days, two weeks apart. We assigned these lizards to two separate groups (1 or 2) based on collection date. Lizards were transported to CIBIO-InBIO, at the

University of Porto, and acclimatised to captivity for two weeks, while being fed every other day with three live mealworms. During the experimental period (two days at a time), lizards were fed the day before trials commenced and at the end of the second day, after trials had finished (Fig. 1). Animals were kept in individual terraria (200 mm W × 300 mm L × 200 mm H) at a temperature of 20-22 °C with constant access to water and a small brick shelter. The room had indirect natural light, as well as artificial lamps set for a photoperiod of 12 hours (08:00 – 20:00). A 50 W heat cable beneath their enclosure created a thermal gradient.

Experimental set up

We separately measured the following behavioural traits: exploration, neophobia, and boldness. All trials were conducted in an experimental arena (320 mm W × 480 mm L × 300 mm H), and repeated three times per individual. Each replicate was separated by one week. We randomly allocated lizards to one of four different batches across the day (batch = 1, 2, 3 or 4), because the number of lizards that could fit in our experimental room was limited. We measured exploration and neophobia on the same day (day 1), and boldness on the following day (day 2; Fig 1). At the beginning of each experimental day, lizards were removed from their enclosure and transferred to the centre of the experimental arena. After the neophobia and boldness tests (on different days), lizards were returned to their home enclosures.

In order to measure distance to a novel object in the neophobia trials, we drew black circles beneath all experimental arenas, and each circle was separated from the next by 2 cm. Lizards always had access to shelters (black plastic containers: 80 mm W × 120 mm L × 50 mm H), with a small opening on one side (40 mm L × 25 mm H). We cleaned all cage materials with 96% ethanol between trials. All trials were remotely video-recorded with CCTV cameras.

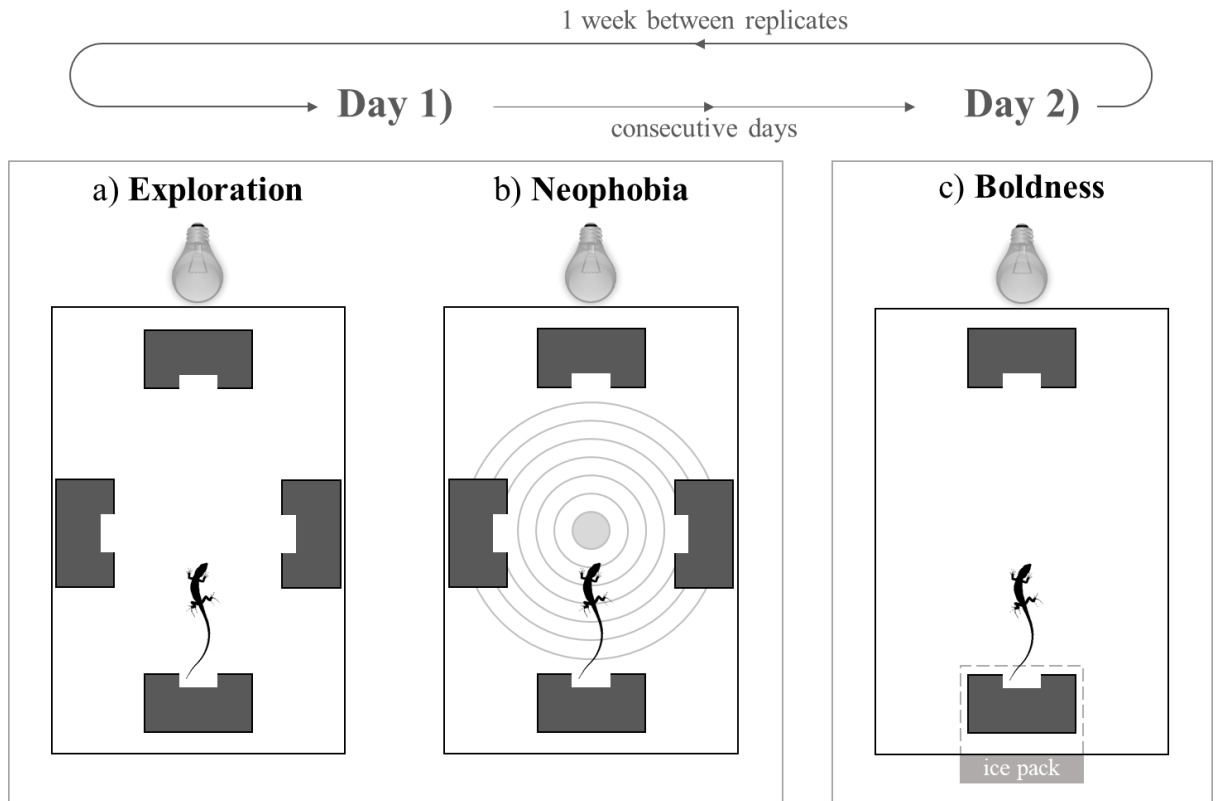


Fig 1. Diagram of the three different behavioural trials and their arena set-up, which were carried out on two consecutive days. Regardless of the trial, all arenas always had a 100W halogen light bulb suspended on one side. Each trial was replicated three times per individual in the same sequence, one week apart. Day 1) refers to the first experimental day in each week during which we measured exploration (a) and neophobia (b). The solid grey circle in the middle of the arena for the neophobia trial was where we placed the novel object. Day 2) refers to the second experimental day in which boldness (c) was measured. All trials took 30 minutes.

Table 1. Behaviours were scored from remotely-recorded videos of the exploration, neophobia, and boldness trials. Explanations of each of the parameters we measured, as well as the response variables used in statistical analyses, and how we interpreted them, are listed.

| Trial | Behaviours scored from videos | Variables |
|--------------------|---|--|
| Exploration | <i>Activity</i> – Time (s) spent moving in the arena (0 to 1800 s) | Exploratory score (PC1) More sedentary with values ↑ More exploratory with values ↓ |
| | <i>Hiding</i> – Time (s) spent inside a shelter (0 to 1800 s) | |
| | <i>Shelter frequency</i> – Number of times entered all shelters (0 to unlimited) | |
| | <i>Shelter number</i> – Number of shelters visited (0 to 4) | |
| Neophobia | <i>Minimum distance</i> – The minimum distance (cm) a lizard would get to the new object during the trial (0 to 14 ⁺ cm) | Minimum distance (Rank transformed) More neophobic with values ↑ More neophilic with values ↓ |
| | | |
| Boldness | <i>Latency to leave shelter</i> – Time (s) to emerge from the cold shelter (0 to 1800 s) | Latency to leave shelter Shyer with values ↑ Bolder with values ↓ |
| | | |

Exploration

We quantified each lizard's exploratory behaviour within a novel environment containing four shelters (Fig. 1a). For each exploration trial (N=3) we used a different substrate – first dark pine bark, then white sand, and then soil. At the beginning of the exploration trial, each lizard was placed under a closed, opaque plastic shelter (circular, 100 mm D × 70 mm H) for 5 minutes, to acclimatise. The arena consisted of four shelters placed along the four sides of the enclosure with the opening facing the centre of the arena. The trial began when we remotely lifted the shelter using wire, so the lizard could not see the researcher, and lasted for 30 minutes. We scored four measures related to exploration: the time spent active (s), time in hiding (s), the frequency an individual entered the shelters (count), and the number of unique shelters visited during the trial (ranges from 0 to 4) (Table

1). We used the program BORIS (Friard and Gamba 2016) to measure the time lizards spent active and in hiding. To create one exploration score for analysis, we performed a principal component analysis (PCA) summarising our four exploration measures using the *princomp* function (Jolliffe 2002) in R version 3.4.2 (R Core Team 2017). Because these variables have different scales, the PCA used a correlation matrix to standardise the data (Jolliffe 2002). The first principal component (PC1) explained 52% of the variance in these four traits, and so we used PC1 in all statistical analyses as the exploration score. The time spent active, frequency of entering a shelter, and the number of unique shelters visited, negatively loaded on PC1, while the time spent hiding positively loaded on PC1 (Table 2). Therefore, as our exploration score decreased, lizards were more exploratory.

Table 2. Exploratory behaviours were combined using a principle component analysis (PCA) to form an exploratory score ($N_{\text{sicula}} = 78$ and $N_{\text{virescens}} = 87$). The first principle component (PC1) explained 52% of the variation in these four behaviours, and was used in statistical analysis as our exploratory score. Higher values of the PC1 reflect less exploratory lizards.

| Exploratory Behaviours | PC1 loadings |
|------------------------|--------------|
| Activity | -0.5026 |
| Hiding | 0.2341 |
| Shelter frequency | -0.5864 |
| Shelter number | -0.5905 |

Neophobia

Once the exploration trial finished, we ushered the lizard into the closed refuge. We then placed a novel object in the centre of the arena (Fig. 1b). After 5 minutes, we lifted the closed shelter using the same method as before. We recorded the lizard's behaviour for 30 minutes and later scored the minimum distance between lizard and the novel object using the circles in the arena. If the lizard contacted the object, it was given a score of 0 cm (Table 1). For each replicate of the neophobia trial ($N=3$) we used a different novel object presented in the order: white non-perfumed candles in foil, yellow cupcake paper, and blue plastic clothes pegs. We chose these objects because lizards are

unlikely to encounter them in the wild, and due to the substantial difference between objects. For the statistical analyses, we applied a rank transformation to our neophobia score to normalize the data (Riley et al. 2017). As the neophobia score decreases, it reflects more neophilic behaviour, because the lizard is closer to the novel object.

Boldness

For this trial, the experimental arena had white paper as a substrate and had one shelter under the heat lamp to create a hot ('optimal') shelter, and another at the opposite end of the enclosure (Fig. 1c). An ice pack was placed under this shelter, to create a cold ('suboptimal') refuge. The mean substrate temperature inside each shelter was $T_{\text{hot}} = 30\text{ }^{\circ}\text{C}$ and $T_{\text{cold}} = 9\text{ }^{\circ}\text{C}$. We placed the lizard in the arena and, after 20 minutes, we started the boldness trial by lifting the hot refuge and, using a nitrile-gloved hand, we gently tapped the lizard repeatedly to scare it inside the cold shelter. Once inside the cold shelter, the hot shelter was placed back in the arena. We then recorded the lizard's behaviour for 30 minutes, and scored boldness as the latency from when the lizard entered the cold shelter until it emerged (Table 1). We believe both species would be similarly impacted by the suboptimal refuge given both inhabit small refuges in the wild and have similar preferred temperatures (Carretero 2015).

Statistical analysis

All analyses were performed in R version 3.4.2 (R Core Team 2017). Prior to analyses we explored our data to ensure it fitted model assumptions using the protocol described in Zuur et al. (2010).

Differences between species

We examined exploration, neophobia, and boldness separately, but used the same analyses. In each model, we tested if the behavioural trait differed between species using a linear mixed effects model (LMM, using the function *lmer* from the *lme4* R package; Bates et al. 2015). These models incorporated the fixed effects of *species* (*P. sicula* or *P. virescens*), the *trial day* (the day the trial

occurred on, with 1 being the first experimental day), the *experimental group* (collection day 1 or 2), and the *batch* (1 to 4). We also initially tested for an interaction between the fixed effect of *species* and *trial day*, which was removed and the model re-run if the effect was non-significant. The continuous predictor variable *trial day* was z-transformed prior to analysis to standardize the variable and facilitate interpretation of interactions if present (Schielzeth 2010). We also included each *lizard's identity* as a random effect (intercept only) in the model to control for dependencies in the data due to repeated behavioural trials on the same lizards. When we plotted our data (see Fig 2), we set the fixed effect of batch to intercept level values, experimental group was set to 2 because it contained a higher number of individuals to ensure better estimation of variance components, and we did not include the variance from the random effect of lizard identity.

Repeatability of behavioural traits

We estimated the consistency of behavioural traits by calculating adjusted repeatability (R_{adj}) with 95% confidence intervals (CIs) for each behavioural trait (exploration, neophobia, and boldness), for each species separately. We used the *rpt* function from the R package *rptR* with 1000 permutations, and accounted for the same covariates used in our LMM models (Nakagawa and Schielzeth 2010). To assess if R_{adj} was significant, we visually examined if 95% CIs for each estimate included 0. We also compared differences in repeatability between species by visually examining overlap in 95% CIs.

Correlations between traits

We also investigated correlations between the behavioural traits for each species separately. This analysis was restricted by the sample size of our study, and we were unable to account for dependencies within our data (i.e., repeated measures of the same individuals) or additional explanatory variables (i.e., experimental group, batch, etc.) in this analysis. However, these analyses may afford some insight into the correlations between behavioural traits in our two study species. We calculated Spearman rank-order correlations between all behavioural traits using the function *cor* in the R package *stats*, and then used the function *cocor.indep.groups* from the R package *cocor* to test

for significant differences between species in trait correlations using Fisher's z -tests (Diedenhofen and Musch 2015).

RESULTS

Behavioural differences between species

Podarcis sicula were significantly more exploratory than *P. virescens* (Table 3a, Fig 2a) and became more exploratory as trials progressed, while *P. virescens* became less exploratory (Table 3a, Fig 2a). Neither group (collection day) nor batch significantly affected lizard exploratory behaviour (Table 3a).

In the neophobia trial, *P. sicula* got significantly closer to the novel object than *P. virescens* (Table 3b, Fig 2b) and was therefore more neophilic than *P. virescens*. We found no effect of trial day or group (collection day) on lizard neophobia score, but we did find a significant effect of batch; batch 4 was significantly less neophobic than batch 1 (Table 3b). We found substantial individual variation in the response to a novel object – some animals never made contact with the novel object and had high values in their minimum distance, while others passed by the object, briefly touching it without paying much attention, and there were also lizards that explored the novel object through tongue flicking and even climbing onto it. During the neophobia trial, 21.8% ($N_{\text{trials}} = 19/87$; $N_{\text{individuals}} = 29$) of *P. virescens* and 37.2% ($N_{\text{trials}} = 29/78$; $N_{\text{individuals}} = 26$) of *P. sicula* explored the novel object.

During the boldness trials, latency to emerge from the cold shelter after being scared was significantly shorter for *P. sicula* than for *P. virescens* (Table 3c, Fig 2c). The model indicated a significant effect of batch, where batch 3 was significantly bolder than batch 1. There was no effect of group (collection day) or trial day (Table 3c).

Table 3. Outcomes of our LMMs for each behavioural trait – a) exploration, b) neophobia, and c) boldness, which included the fixed and random effects indicated below.

When the interaction effect was non-significant, which is indicated with a “-”, we re-ran the model without this effect. Significant results are presented in bold.

| | a) Exploration | | | | b) Neophobia | | | | c) Boldness | | | |
|---|-------------------------------|--------------|---------------|------------------|-------------------------------|--------------|---------------|------------------|-------------------------------|----------------|---------------|------------------|
| | $N_{obs} = 165, N_{ind} = 55$ | | | | $N_{obs} = 165, N_{ind} = 55$ | | | | $N_{obs} = 165, N_{ind} = 55$ | | | |
| Fixed effects | β | SE | t-value | p | β | SE | t-value | p | β | SE | t-value | p |
| Intercept | -1.089 | 0.298 | -3.662 | <0.001 | -0.190 | 0.180 | -1.054 | 0.292 | 650.455 | 172.600 | 3.769 | <0.001 |
| Species (<i>virescens</i> ; REF = <i>sicula</i>) | 1.451 | 0.254 | 5.719 | <0.001 | 0.582 | 0.151 | 3.858 | <0.001 | 561.030 | 143.234 | 3.917 | <0.001 |
| Trial Day | -0.337 | 0.121 | -2.797 | 0.005 | -0.042 | 0.054 | -0.777 | 0.437 | 32.293 | 32.925 | 0.981 | 0.327 |
| Group (2; REF = 1) | 0.328 | 0.263 | 1.244 | 0.213 | 0.047 | 0.157 | 0.297 | 0.766 | 139.666 | 151.958 | 0.919 | 0.358 |
| Batch (2; REF = 1) | 0.135 | 0.219 | 0.613 | 0.540 | -0.058 | 0.142 | -0.407 | 0.684 | -10.293 | 116.764 | -0.088 | 0.930 |
| Batch (3; REF = 1) | 0.248 | 0.266 | 0.933 | 0.351 | 0.056 | 0.170 | 0.328 | 0.743 | -245.284 | 124.279 | -1.974 | 0.048 |
| Batch (4; REF = 1) | 0.212 | 0.256 | 0.827 | 0.409 | -0.566 | 0.167 | -3.399 | 0.001 | -75.716 | 131.157 | -0.577 | 0.564 |
| Species*Trial Day (<i>virescens</i> ; REF = <i>sicula</i>) | 0.597 | 0.166 | 3.592 | <0.001 | - | - | - | - | - | - | - | - |
| Random effects | σ^2 | SE | | | σ^2 | SE | | | σ^2 | SE | | |
| Lizard identity | 0.470 | 0.053 | | | 0.139 | 0.029 | | | 207288.000 | 35.445 | | |
| Residual | 1.104 | 0.082 | | | 0.472 | 0.054 | | | 174750.500 | 32.541 | | |

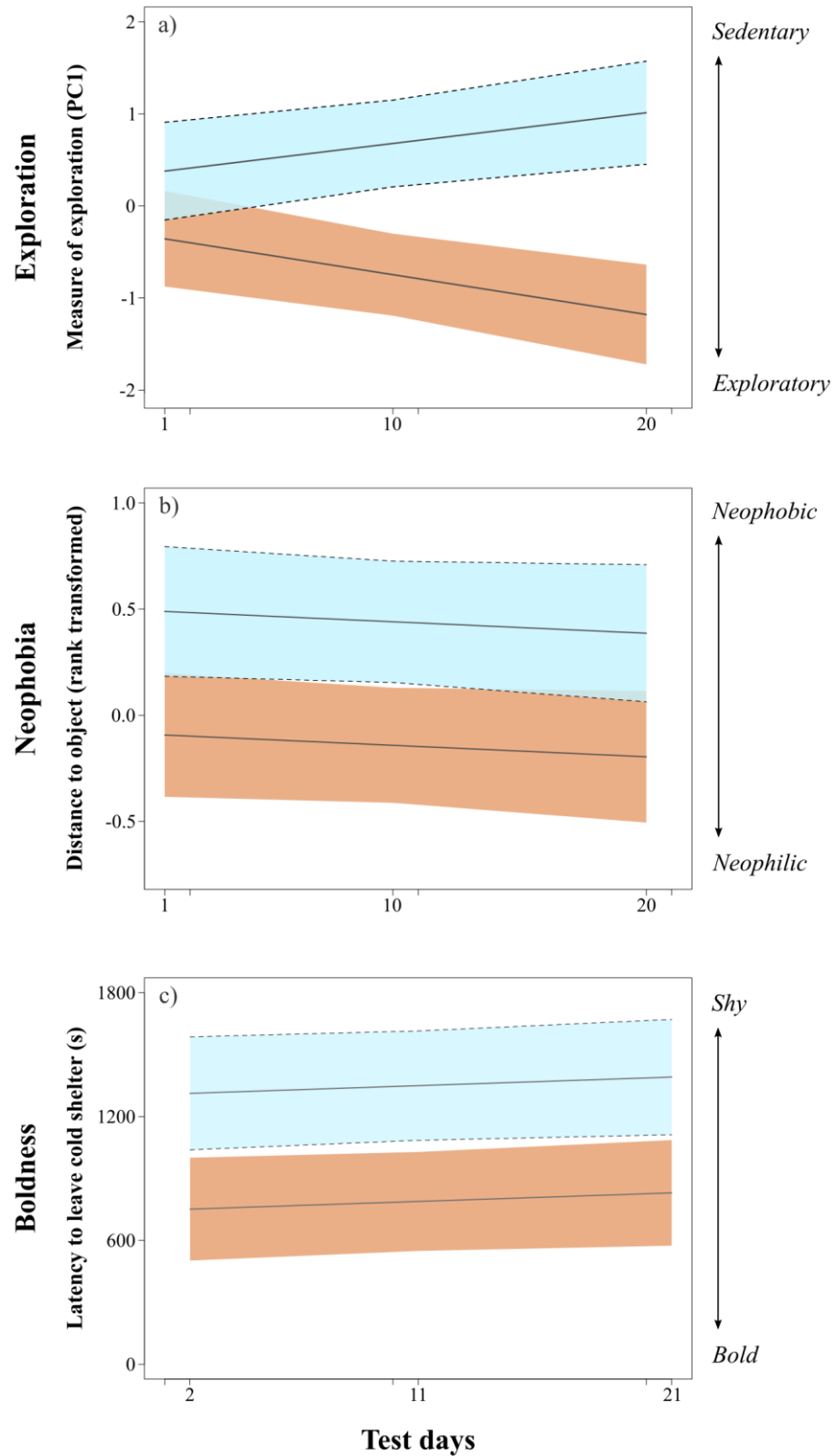


Fig 2. Behavioural differences between the invasive *P. sicula* and the native *P. virescens* for: a) exploration, b) neophobia, and c) boldness. *P. sicula* (shaded in orange with grey solid lines) is more exploratory, neophilic, and bolder than *P. virescens* (shaded in blue with grey dashed lines). We plotted fitted lines predicted from our linear mixed effect models with 95% confidence intervals (shaded polygon).

Repeatability of behavioural traits

Podarcis virescens was significantly repeatable in all three behavioural traits (Fig. 3), whereas *P. sicula* was significantly repeatable in their boldness (Fig. 3). The species were not significantly different from each other in the repeatability of behavioural traits (Fig. 3).

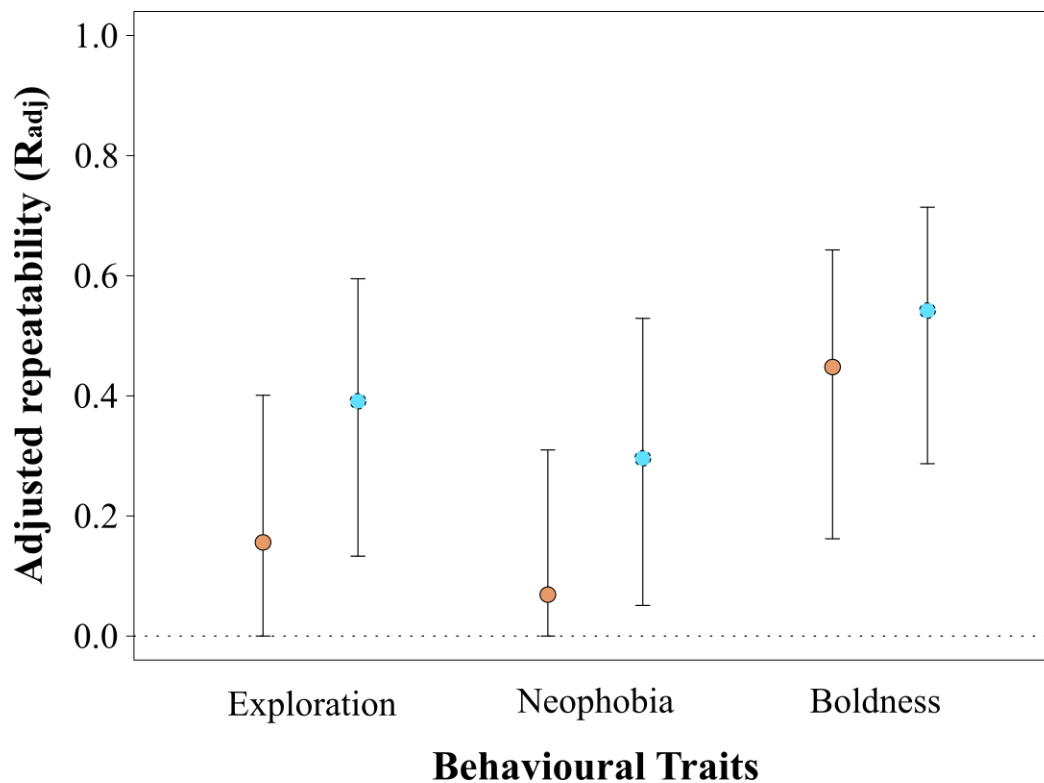


Fig 3. Adjusted repeatability (R_{adj}) and 95% CIs of behavioural traits (exploration, neophobia, and boldness) for the invasive *P. sicula* and the native *P. virescens*. *P. sicula* ($N_{obs} = 78$, $N_{ind} = 26$) is represented by orange circles with solid outline, while *P. virescens* ($N_{obs} = 87$, $N_{ind} = 29$) is represented by blue circles with dashed outline. *Podarcis sicula* did not exhibit significant repeatability in exploratory or neophobia score (R_{adj} 95% CIs include 0), but all other R_{adj} are significant.

Correlations between traits

The behavioural traits measured in *P. virescens* were significantly correlated; individuals that exhibited more exploratory behaviour were also bolder and less neophobic (Table 4). We did not find any significant correlation between *P. sicula* behavioural traits (Table 4), and the difference between species' behavioural trait correlations were all non-significant (Table 4).

Table 4. Spearman rank-order correlations and 95% Cis (in parentheses) between all behavioural traits for each species, *P. sicula* and *P. virescens*, separately. We also present the outcomes of a Fisher's z-test that was used to compare behavioural trait correlations between species. Significant results are in bold.

| | Exploration vs. Boldness | Boldness vs. Neophobia | Neophobia vs. Exploration |
|---------------------|--------------------------------|--------------------------------|--------------------------------|
| <i>P. sicula</i> | 0.0793 (-0.1439, 0.3039) | 0.1481 (-0.1023, 0.3676) | 0.1917 (-0.0358, 0.4041) |
| <i>P. virescens</i> | 0.2452 (0.0432, 0.4250) | 0.3043 (0.1045, 0.4949) | 0.3708 (0.1534, 0.5501) |
| Species comparison | $z = 1.32, p = 0.1853$ | $z = 1.04, p = 0.2989$ | $z = 1.23, p = 0.2191$ |

DISCUSSION

Overall, our predictions for the behavioural differences between an invasive and a native species were supported. The invasive species, *P. sicula*, was more exploratory, bolder, and less neophobic than the native *P. virescens*. *Podarcis virescens* were highly repeatable in their behaviours, while only boldness of *P. sicula* was repeatable. The native *P. virescens* also exhibited correlations between all behavioural traits, with more exploratory individuals also being bolder and less neophobic. In contrast, *P. sicula* did not exhibit any significant correlations between its behavioural traits.

Being bolder, more exploratory, and neophilic likely enhances the ability of *P. sicula* to be successful during all aspects of the invasion process. For example, bolder and more exploratory behaviour may increase an invader's likelihood to enter into a transport vector, and thereby colonise a new environment (Chapple et al. 2012, Griffin et al. 2016). These behaviours might have been similarly expressed and important for *P. sicula* when they were introduced 20 year ago, as these behavioural

traits are likely heritable (Gruber et al. 2017b, Réale et al. 2007). We cannot exclude the possibility that selection might have acted on these behavioural traits during the invasion process, leaving only the bolder, more exploratory and neophilic individuals. Nevertheless, the differences between traits we found in this study might favour the invasive species when it has to compete for food and habitat (by more easily finding and exploiting new resources, such as food and shelter sites), and increase its likelihood of interacting with other lizards (by being bolder for example), which can promote aggressive encounters with native species (Candler and Bernal 2015, Gruber et al. 2017a, Rehage and Sih 2004, Short and Petren 2008, Sol et al. 2002). Indeed, the displacement of *P. virescens* from gardens inhabited by *P. sicula* (Ribeiro 2017), or of other *Podarcis* sp. native to other invaded sites, may be explained in part by higher levels of aggression in this species (Downes and Bauwens 2002), which can result in competitive exclusion of native species (Nevo et al. 1972, Valdeón et al. 2010).

Podarcis virescens was consistent in all behavioural traits, while *P. sicula* was only consistent in their boldness. This was a similar result to a study of hymenopterans, where the native wasp *V. crabro* was repeatable for activity, boldness and exploration, but the invasive *V. velutina* was not (Monceau et al. 2015). The invasive *P. sicula* may benefit from being more plastic in its behaviour because invasive species in general have to respond to changing, novel environments (Griffin et al. 2016). *Podarcis sicula* is usually unintentionally transported to new locations (Carretero and Silva-Rocha 2015), and very successful at adapting to new conditions (Vervust et al. 2007, Herrel et al. 2008), and behavioural plasticity may thus partly explain this species' invasion success. Interestingly, *P. sicula* did exhibit repeatability in boldness, which potentially highlights the importance of boldness in all stages of the invasion process in this species. For example, bold individuals may also be more likely to be transported outside their native range by virtue of the fact that they are more likely to enter containers or vessels being prepared for transport (e.g. olive trees; Rivera et al. 2011, Silva-Rocha et al. 2012). After introduction in a novel location, bolder individuals may gain greater access to resources and do better in social interactions (Monceau et al. 2015, Pintor et al. 2008, Short and Petren 2008). Moreover, *P. sicula* usually invades urbanised locations perhaps because they are in or near transport hubs (the introduced population in Lisbon inhabits city gardens), and boldness confers an advantage in urban environments because it can translate into higher foraging efficiency (Short and Petren 2008).

Behavioural traits of *P. virescens* were correlated with more exploratory individuals being also bolder and less neophobic, which suggests a possible behavioural syndrome in this native species (Sih et al. 2004). However, the same was not true for *P. sicula*, for which we did not find any correlations between their behavioural traits. It is important to note that the correlations between behavioural traits we found in this study should be interpreted with caution, because we did not control for dependency among variables or additional sources of variation. It is also important to consider both within- and between-individual correlations in behavioural traits, to understand the selective forces acting on behavioural traits within a population and their evolutionary significance (Dingemanse and Wolf 2013, Sih et al. 2012). Nevertheless, the lack of correlation between traits, allied with the overall inconsistency in *P. sicula*'s repeatability, may be advantageous during biological invasions. Variation in behavioural traits within a population increases the likelihood of success in fluctuating environments and novel habitats and allow for a population's persistence in novel environments (Dingemanse and Wolf 2013, Sih et al. 2012). Likewise, correlations between behavioural traits constrain a population, because if selection acts on one trait, correlated behaviours are also likely to be affected (Sih et al. 2012).

In conclusion, we show that congeneric invasive and native lizard species differed in key behavioural traits: exploration, neophobia, and boldness, traits that could promote the invasion success of *P. sicula*. Also, it is likely that these behavioural traits are important for the success of other introduced *P. sicula* populations given that these populations share the same long-range transportation and establishment pattern (CABI 2018, Carretero and Silva-Rocha, 2015). Increasingly, behavioural mechanisms are being appreciated as playing an important role in determining invasion success (Chapple et al. 2012). We also suggest that comparisons between closely related species that are variable in their invasive ability may provide important insights into the relationship between plasticity and personality and their relative roles in determining invasive success.

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ETHICS: Experimental protocols were approved by the Macquarie University Animal Ethics Committee (ARA 2017/004). Our fieldwork and research was also approved by the Portuguese Institute for Conservation of Nature and Forests (ICNF) (License 428/2017/CAPT).

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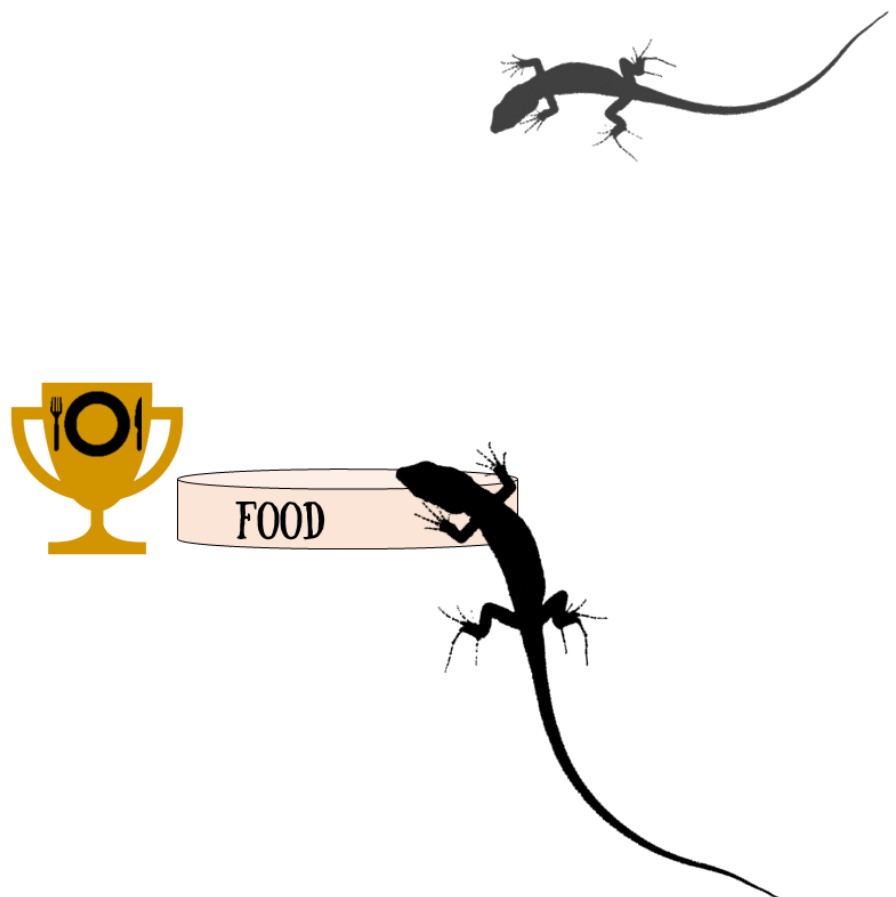
Chapter IV

Getting ahead:

Exploitative competition by an invasive lizard

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Manuscript submitted



Getting ahead:

Exploitative competition by an invasive lizard

ABSTRACT

Biological invasions are a global contemporary threat because invasive species have substantial negative economic and ecological impacts. Invasive species can outcompete native species through two main different mechanisms: interference competition (direct negative interactions such as aggression) and/or exploitative competition (indirect negative interactions resulting from species using the same limited resources, such as food). Understanding these competitive mechanisms are vital for determining their impact on native species and for understanding how it influences the invasive process. The invasive Italian wall lizard *Podarcis sicula* was introduced into Lisbon (Portugal) 20 years ago and is believed to be displacing the native green Iberian wall lizard *Podarcis virescens*. We experimentally tested for competition by establishing heterospecific (1 pair of each species) and conspecific (2 pairs of same species; control) treatments under captive conditions in which each enclosure contained a high- and low-quality refuge. Lizards were fed from set food dishes every other day. We analysed if species showed interference competition (aggressive behaviours, stealing food, and shelter exclusion) or exploitative competition (tolerance between species, but differences in food consumption efficiency). We found evidence for exploitative competition: the invasive *P. sicula* arrived first at food stations and consumed more food than *P. virescens*. We suggest that exploitative competition may in part explain the observed displacement of *P. virescens* from contact areas with *P. sicula*.

KEYWORDS

Podarcis sicula; biological invasions; sympatry; native species

INTRODUCTION

Biological invasions are a global contemporary problem because invasive species have substantial negative impacts on economies, native species, and ecosystems (Crooks 2002, Hulme 2007, Walsh et al 2016). An invasive species can have devastating effects on native species by, for example, affecting their development (Kupferberg 1997, Carmo et al 2018), being more aggressive (Holway 1999, Downes and Bauwens, 2002), by hybridizing (Huxel 1999), consuming more food (Short and Petren 2008, Pintor and Sih 2009) and even through predation (Pintor et al 2009). The different evolutionary history and experience of invasive species sometimes brings together traits that give them an edge over native species adapted to local conditions (Sax and Brown 2000). These traits can ultimately lead to the displacement or even eradication of native species. Understanding the direct effects and mechanisms through which an invasive outcompetes a native species, is of major importance in managing and ameliorating their impacts.

The idea that invaders can have an advantage over natives due to their competitive ability is a long-standing one (Elton 1958). Competition between two species can happen through two main processes – interference and/or exploitation (Miller 1967). Interference competition refers to direct negative interactions resulting from aggressive behaviour. With interference competition, an invasive species may be more aggressive, steal food, or exclude others from resources (Pintor and Sih 2009, Michaud 2002, Rowles and O’Dowd 2007). Exploitative competition refers to indirect negative interactions resulting from species using the same, limited resources, such as food or water. With exploitative competition, an invasive species does not necessarily interact with another individual or species, but has an advantage in accessing resources, for example by more efficiently locating and consuming food (Petren and Case 1996, Human and Gordon 1996). An invasive species that combines these two strategies can pose a greater threat to native species (Case et al 1994, Human and Gordon 1996, Amarasekare 2002). For example, the invasive Argentine ant (*Linepithema humile*) can exhibit both types of competition towards native ants, by restricting their foraging activity while simultaneously also preying on native ant queens, which prevents the establishment of new native colonies (Human and Gordon 1996). Nevertheless, either type of competition can independently result in the displacement from optimal habitats or complete exclusion of other species (Grether et al 2017).

The Italian wall lizard, *Podarcis sicula*, is a small lacertid native to the Italian Peninsula and Adriatic coast, but largely introduced along the Mediterranean Basin and even Britain and North America (CABI 2018, Kraus 2009, Carretero and Silva-Rocha 2015). It can become quickly established in new areas (Burke et al 2002, Vervust et al 2007) and impacts native lizards, leading to their eradication or displacement to poorer habitats (Nevo et al 1972, Capula et al 2002, Downes and Bauwens 2002, Herrel et al 2008). The only experimental study directly testing the mechanism underlying competition, found that *P. sicula* displaces the sympatric Dalmatian wall lizard, *Podarcis melisellensis*, through interference competition (Downes and Bauwens 2002). In staged encounters between newborns of the two species, *P. sicula* were more dominant and aggressive. Also, when high-quality microhabitats were available, *P. sicula* used them more efficiently than *P. melisellensis*, and heterospecific pairs were less likely to simultaneously occupy the same patch, compared to conspecific pairs (Downes and Bauwens 2002). However, even within the same invasive species, different populations might use different competitive strategies depending, for example, on the context of local conditions (Amarasekare 2002). The employed competition strategy can depend on the dynamics, availability, and abundance of resources (Amarasekare 2002, Perkins and Nowak 2013), or on the specific traits that differ between the invasive and native species (Persson 1985).

A *P. sicula* population in Lisbon (Portugal) was unintentionally introduced from North Italy in 1998, and is currently a high-density population in an urban environment (González de la Vega et al 2001, Silva-Rocha et al 2012, CABI 2018). It is likely that *P. sicula* is excluding the native green Iberian wall lizard *Podarcis virescens* to surrounding urban gardens since both species live in close sympatry but not in syntopy (Ribeiro 2017), and given that the displacement of native *Podarcis* species after arrival of *P. sicula* is a common phenomenon (e.g. Nevo et al 1972, Capula et al 2002, Herrel et al 2008). Both *P. sicula* and *P. virescens* are diurnal and feed mainly on terrestrial invertebrates, and occupy similar shelters and habitat types such as walls, bushes, and sandy substrates (reviewed in Ribeiro 2017). We tested the hypothesis that *P. sicula* compete with native *P. virescens* by staging interactions in a captive, controlled environment. We predicted that the invasive *P. sicula* would either exhibit interference competition and use aggressive behaviour to steal food (kleptoparasitism) or exclude the native *P. virescens* from food and shelter, and/or use exploitative competition by arriving at food sources first and consuming food more efficiently.

MATERIAL AND METHODS

Collection and acclimatization

We collected 16 *P. sicula* (8 males and 8 females) and 16 *P. virescens* (8 males and 8 females) from the wild during spring, at least 1.6 km apart at Parque das Nações, Lisbon (38.762131, -9.094451 and 38.774001, -9.091770, respectively). Lizards were transported to facilities at CIBIO-InBIO, University of Porto, and acclimatized to captivity for two weeks. During this acclimatization period, animals were kept in individual terraria (200 mm W × 300 mm L × 200 mm H), with indirect natural light and under an artificial light:dark 12:12 h photoperiod (08:00 – 20:00 h), with room temperatures of 20-22 °C during the day. A 50 W heat cable was beneath part of the terrarium, creating a thermal gradient. Lizards had continuous access to water and a small brick shelter, and were fed every other day with three live mealworms.

Experimental protocol

We staged interactions between the two species to test for their competitive abilities. We had two experimental treatments: lizards housed with conspecifics (two male and two female *P. sicula* or two male and two female *P. virescens*) or with heterospecifics (one male and one female of each species). In the wild, *P. sicula* is naturally larger than *P. virescens* and therefore, in the heterospecific treatment (species paired together), *P. sicula* was always larger than *P. virescens*. To minimize this effect we matched individuals that were closest in size (snout-vent length; SVL); the descending order of body size (SVL) for the heterospecific treatment was *P. sicula* male (the largest), followed by the female, *P. virescens* male, and finally *P. virescens* female (the smallest; refer to Table S1 in the supplementary material for mean differences in SVL among treatments). Lizards were thus allocated according to SVL (N = 4 per group) in large experimental tubs (1200 mm W x 1000 mm L x 500 mm H). Trials were one week in duration and were carried out over three consecutive weeks; the conspecific treatment took place within the first two weeks (in each week, four tubs had *P. sicula*, and the other four tubs had *P. virescens*), and the heterospecific treatment took place in the third week (all eight tubs had both species). For clarification, please see Fig S1 in the supplementary material. During the conspecific treatment (week 1 and 2) none of the lizards were held with the same lizard more than

once. However, in the heterospecific treatment all species pairs had been together previously during the conspecific treatment (all species pairs had been together in week 1). At the end of each week, lizards were removed from the tubs, spent overnight in their individual terrarium, and placed into a new clean tub in the following day.

We allowed the lizards to freely interact for one week. During this time we scored the lizard's activity, use of space, feeding behaviour, basking tolerance, and social interactions. We provided lizards with a high- and low-quality shelter, constant access to water, and with food every other day (Fig 1). We used a medium-grained white sand as a substrate, because both species sometimes live in sandy habitats. Each tub had a heat and light source from 10:00-16:00 h every day, placed over the good shelter. The high-quality shelter was maintained at 28-30 °C, reflecting the preferred temperatures of both species (ca. 30 °C; Carretero 2015), while the low-quality shelter was maintained at 20-22 °C. All lizards were individually marked with white TESA® tape to allow easy visual identification. We used four codes: 1 bar, 2 lines, 3 stripes, and 2 dots (Fig. 1). All tubs were remotely video-recorded continuously with CCTV cameras, to minimise any disturbance. The codes provided to the lizards were clearly distinguishable in the videos, and allowed posterior blind video scoring by a single individual. We cleaned tubs with 96% ethanol and the sand substrate was replaced between each experimental week.

All lizards were measured for SVL (± 0.01 mm), weighed (± 0.01 g), and individually marked with the white tape before the experiment started. At the end of each week, all lizards were again weighed and recoded with tape. We also checked for scars between each week, but only three *P. sicula* females showed new ventral scars after the conspecific treatments, which resulted from attempted copulations; consequently, we did not include the number of scars in our statistical analyses.

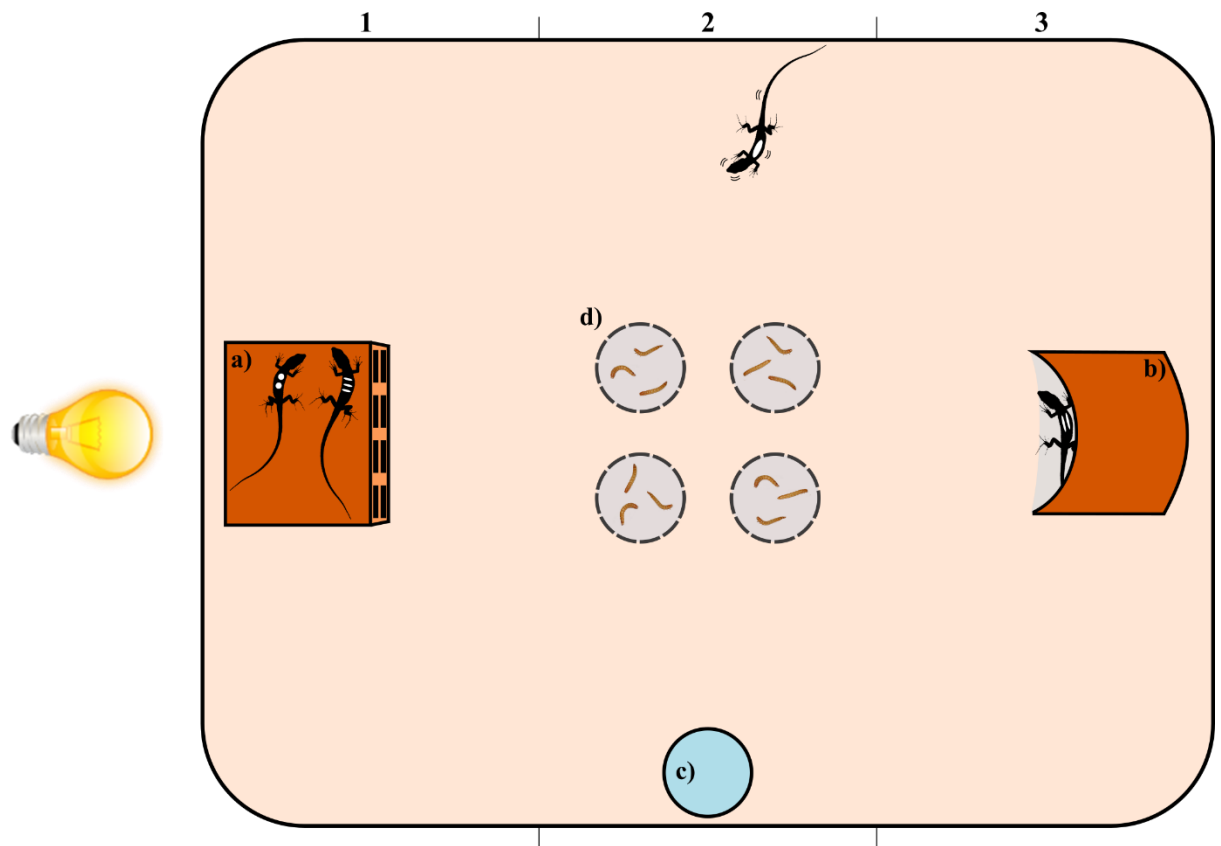


Fig 1. Schematic representation of the experimental tub. We created a single high-quality refuge consisting of a brick with eight small crevices with a heat and light source above (a), and a low quality refuge consisting of a brick with only one large opening with no heat or light source (b). A water bowl was placed between both shelters (c). On feeding days (every other day), the food (12 live mealworms) was placed in four transparent dishes (d). In this example, lizards with 2 *dots* and 3 *stripes* are “inactive” in area “1”, and not touching each other; the lizard with 1 *bar* is “moving” in area “2”, and the lizard with 2 *lines* is “hiding” in area “3”.

Video scoring

We scored videos by recording behaviours every 15 minutes (as in Downes and Bauwens 2002) from 10:00 – 16:30 h, resulting in a total of 27 sample points per lizard per day. We scored lizard activity (hidden or active), location in the tub (1, 2, or 3), and number of contacts between two lizards. The number of contacts was scored as the number of times two lizards were in physical contact (a contact would be scored when two lizards' SVL were in contact) while basking on the high-quality refuge. Every 15 minutes we paused the video and recorded this information for each lizard. Activity and location provided information about an individual's sheltering behaviour and use of space, while the number of contacts provided information about their social tolerance to other lizards while basking on the high-quality shelter.

By continuously observing the videos, we recorded the amount of food each lizard ate, the order that lizards arrived at the food dishes, and if they stole mealworms from other lizards (kleptoparasitism). We also continuously scanned all videos over the entire experimental period and recorded any instances of aggression. See Table 1 for details on the scored behaviours and Table 2 for predictions of behaviour associated with interference or exploitative competition.

Table 1. Behaviours studied during the experiment and how these variables were summarized for use in statistical analyses.

| Behaviour | Parameters | Scoring | Variables |
|-----------------------------|---|---|---|
| Sheltering behaviour | Activity - If the lizard is concealed (in refuge) or visible in the tub (moving or inactive). | Hidden or active | Sheltering behaviour – we used the number of times “hidden” out of total observations |
| | Location - The area of the tub was divided in three equal areas, where 1 corresponds to the area that encompasses the high-quality shelter (also light and heat), 3 to the area with the low quality shelter, and 2 the neutral area in the middle, and we observed where the lizard was in the tub at each time point. | Area 1, 2, or 3 | Use of high quality area – we used the number of times in “area 1” out of total observations |
| Feeding behaviour | Consumption of food - Number of mealworms each lizard ingested on each feeding day | From 0 to 12 | Amount of food |
| | Weight - Variation of the lizard weight (g) before the experiment start (initial weight) and at the end of each week (final weight) | Weight (± 0.01 g) | Weight – we standardized weight: $\frac{(\text{final weight} - \text{initial weight})}{\text{initial weight}} \times 100$ |
| | Arrival order to food - The order in which each lizard arrived to the food dishes on each feeding day | 1, 2, 3, or 4 | Order to food |
| | Stealing food - If the lizard stole food from other | Failed or successful | NA (described qualitatively) |
| Social interactions | Social tolerance - If lizard is in contact with another lizard, when basking on the good shelter | In contact with a conspecific or heterospecific | Number of contacts |
| | Aggression - Any aggressive act recorded during 08:00 – 19:00 h | Aggression for food, or unrelated to food | NA (described qualitatively) |

Table 2. Predictions in support of interference and exploitative competition for behaviour and the use of space in the heterospecific treatment, when both species were together. *Podarcis sicula* is the invasive species and *P. virescens* the native species.

| Predictions for interference competition | |
|--|---|
| Sheltering behaviour | <i>P. virescens</i> will hide more than <i>P. sicula</i> |
| Use of high quality area | <i>P. virescens</i> will use high-quality area less than <i>P. sicula</i> |
| Stealing food | <i>P. sicula</i> will more likely steal food from <i>P. virescens</i> |
| Aggression | <i>P. sicula</i> will more likely be aggressive towards <i>P. virescens</i> than the opposite |
| Predictions for exploitative competition | |
| Consumption of food | <i>P. sicula</i> will consume more food than <i>P. virescens</i> |
| Weight | <i>P. sicula</i> will gain more weight than <i>P. virescens</i> |
| Arrival order to food | <i>P. sicula</i> will arrive to food before <i>P. virescens</i> |
| Social tolerance | Both species will tolerate one another |

Statistical Analyses

Prior to statistical analyses, we explored our data to ensure it fitted all model assumptions (such as no influential outliers, no collinearity between predictor variables within a model, data integrity) following Zuur et al (2010).

We did not include SVL in any of the models, because SVL exhibited high collinearity with our factorial variables of species and sex. Specifically, *P. sicula* was larger than *P. virescens* ($B = -9.74$, $t = -41.15$, $P < 0.001$; results from a linear model performed using the *lm* function in the R package *stats* with sex, species, and the interaction between the two as fixed effects), and males were larger than females ($B = 8.473$, $t = 35.67$, $P < 0.001$). Also, there was a significant interaction effect between species and sex ($B = -6.678$, $t = -19.88$, $P < 0.001$); specifically, *P. virescens* females were the smallest, followed by *P. virescens* males, *P. sicula* females, and male *P. sicula* (i.e., largest). All analyses were performed in R version 3.4.2 (R Core Team 2017). For all models, α was set at 0.05, and we always ensured models fulfilled their assumptions. To create contrasts between relevant

predictor variables, and correct these multiple comparisons with a post-hoc Tukey HSD multiplicity adjustment, we used the *lsmeans* R package with the function *lsmeans* (Lenth 2016).

Sheltering behaviour and use of high-quality area

To examine differences in the proportion of observations a lizard was hiding across treatments, we used a generalized linear mixed effect model (GLMM) with the function *glmer* in the *lme4* R package (Bates et al 2015). This response variable was proportional (between 0 and 1), so we used a binomial distribution (with a logit link; Crawley 2012). The model included the fixed effects of treatment (conspecific or heterospecific), species (*P. sicula* or *P. virescens*), sex (female or male), and experimental day (continuous from 1 to 7). We initially included the interaction between treatment and species, in this and all models containing both these variables, and then, if the interaction was not significant, we removed the interaction and re-ran the model. We also included lizard identity as a random factor, to control for dependencies in our data due to repeated measures of the same individual over experimental days and treatments. Additionally, we included tub and week as other random factors, to control for dependencies in these variables within our study. We used a GLMM with the same distribution and variables as the model described above, to determine if the proportion of observations spent in the high-quality area differed between treatment, species, sex, or experimental day.

Consumption of food, order of arrival to food dish, and weight

To test if the number of food items eaten differed between treatment and species, we used a GLMM with a Poisson distribution. The model included the same variables as described above, but the experimental day variable differed slightly, varying from 1 to 3, because it only included day in which lizards were fed. To analyse the order of arrival to food dish we used only data from the heterospecific treatment (in week 3). We used a GLMM with a Poisson distribution to analyse if the order in which an individual arrived at the food dishes (1, 2, 3, or 4) was affected by species (*P. sicula* or *P. virescens*), sex (female or male), or experimental day (from 1 to 3). We also included lizard identity,

and tub as random factors in this model. Finally, we used a Gaussian linear mixed-effect model (LMM) to examine if lizard weight was influenced by treatment, species, sex, or by the interaction treatment \times species. We incorporated lizard identity, tub, and week as random factors.

Social tolerance

We used a GLMM with a Poisson distribution to analyse whether the frequency with which lizards were in contact varied by treatment and experimental day (continuously varying from 1 to 7). In this model, “treatment” included three levels: the conspecifics treatment with *P. sicula*, the conspecifics treatment with *P. virescens*, and the heterospecific treatment. This difference was because this interaction data is not tied to a particular individual, or species, and instead is a summary across each experimental day. We also included tub and week as random factors in our model. To account for overdispersion in this model, we also added an observation-level random effect.

RESULTS

In all treatments, all lizards spent the night inside crevices provided by the high-quality shelter, with the exception of one night where one lizard spent the night exposed (a female *P. virescens* housed with conspecifics in week 2). Videos did not reveal in which of the eight crevices the lizards would refuge in overnight, but they often slept in the same crevice (IDM, personal observations before 9 am). Lizards would thus often aggregate overnight even though there were unoccupied crevices.

Sheltering behaviour and use of high-quality area

As the experiment progressed, the lizards hid less (Table 3a). We found a significant main effect of species (Table 3a), while sheltering behaviour in the heterospecific treatment was not different between species (Table 4a). We also found a significant interaction on the proportion of observations spent hiding between species and treatment (Table 3a): when housed with a conspecific, *P. sicula* hid more often than *P. virescens* (Table 4a). We detected no effect of experimental day, sex, treatment, or species on the proportion of observations a lizard was within the high-quality area (Table 3b).

Consumption of food, order of arrival to food dish, and weight

Lizards ate more as the experiment progressed, and males ate more than females (Table 3c). We detected a significant main effect of treatment, but no significant main effect of species (Table 3c). We also found a significant interaction effect on the amount of food eaten between species and treatment (Table 3c): in the conspecific treatment, *P. sicula* and *P. virescens* ate the same amount of food, but in the heterospecific treatment *P. sicula* ate significantly more than *P. virescens* (Table 4b; Fig 2). The order of arrival at the food dish in the *heterospecific* treatment was not significantly affected by sex or experimental day (Table 3e), but *P. sicula* approached the food dish significantly sooner than *P. virescens* (Table 3e; Fig 3). Also, we found a significant main effect of treatment on the lizards' weight, and also of the interaction between treatment and species (Table 3d). Specifically, *P. sicula* increased in weight after the heterospecific treatment, compared to the conspecific treatment (Table 4c). In contrast, *P. virescens* maintained their weight between the conspecific and the heterospecific treatments (Table 4c).

Table 3. Results of our statistical analyses. Reference levels for all fixed factors are shown in parentheses. When predictor variables were not used in the model this is indicated with “NA”, and when an interaction was initially included but removed from final model for not being significant it is indicated with “-”. Significant values are indicated in bold.

| a) Sheltering behaviour <i>N_{obs}</i> = 672, <i>N_{ind}</i> = 32 | | | | | b) Use of high quality area <i>N_{obs}</i> = 672, <i>N_{ind}</i> = 32 | | | | |
|---|---------------|--------------|-----------------|------------------|---|---------------|--------------|-----------------|------------------|
| | β | SE | <i>t</i> -value | <i>p</i> | | β | SE | <i>t</i> -value | <i>p</i> |
| Intercept (<i>P. sicula</i> , Female, & Conspecific) | -0.595 | 0.193 | -3.081 | 0.002 | Intercept (<i>P. sicula</i> , Female, & Conspecific) | -0.080 | 0.029 | -2.718 | 0.007 |
| Day | -0.043 | 0.008 | -5.775 | <.0001 | Day | 0.003 | 0.005 | 0.613 | 0.540 |
| Species (<i>P. virescens</i>) | -0.628 | 0.133 | -4.728 | <.0001 | Species (<i>P. virescens</i>) | -0.022 | 0.021 | -1.016 | 0.309 |
| Sex (Male) | -0.199 | 0.131 | -1.521 | 0.128 | Sex (Male) | -0.004 | 0.021 | -0.166 | 0.868 |
| Treatment (Heterospecific) | -0.192 | 0.192 | -0.999 | 0.318 | Treatment (Heterospecific) | 0.004 | 0.023 | 0.184 | 0.854 |
| Species (<i>P. virescens</i>) : Treatment (Heterospecific) | 0.426 | 0.065 | 6.584 | <.0001 | Species (<i>P. virescens</i>) : Treatment (Heterospecific) | - | - | - | - |
| c) Amount of food <i>N_{obs}</i> = 288, <i>N_{ind}</i> = 32 | | | | | d) Weight <i>N_{obs}</i> = 96, <i>N_{ind}</i> = 32 | | | | |
| | β | SE | <i>t</i> -value | <i>p</i> | | β | SE | <i>t</i> -value | <i>p</i> |
| Intercept (<i>P. sicula</i> , Female, & Conspecific) | 0.673 | 0.186 | 3.610 | <.0001 | Intercept (<i>P. sicula</i> , Female, & Conspecific) | 5.022 | 2.376 | 2.113 | 0.035 |
| Food day | 0.028 | 0.044 | 0.631 | 0.528 | Food day | NA | NA | NA | NA |
| Species (<i>P. virescens</i>) | -0.260 | 0.176 | -1.479 | 0.139 | Species (<i>P. virescens</i>) | 4.381 | 2.763 | 1.586 | 0.113 |
| Sex (Male) | 0.396 | 0.168 | 2.360 | 0.018 | Sex (Male) | -0.183 | 2.705 | -0.068 | 0.946 |
| Treatment (Heterospecific) | 0.383 | 0.142 | 2.699 | 0.007 | Treatment (Heterospecific) | 3.812 | 1.189 | 3.205 | 0.001 |
| Species (<i>P. virescens</i>) : Treatment (Heterospecific) | -0.724 | 0.164 | -4.416 | <.0001 | Species (<i>P. virescens</i>) : Treatment (Heterospecific) | -3.645 | 1.682 | -2.168 | 0.030 |
| e) Order to food (Heterospecific treatment only) <i>N_{obs}</i> = 92, <i>N_{ind}</i> = 32 | | | | | f) Number of contacts <i>N_{obs}</i> = 168, <i>N_{ind}</i> = 32 | | | | |
| | β | SE | <i>t</i> -value | <i>p</i> | | β | SE | <i>t</i> -value | <i>p</i> |
| Intercept (<i>P. sicula</i> , Female) | 0.706 | 0.206 | 3.429 | 0.001 | Intercept (Invasive) | -0.214 | 0.358 | -0.597 | 0.551 |
| Food day | 0.024 | 0.083 | 0.288 | 0.773 | Day | 0.141 | 0.038 | 3.749 | <.0001 |
| Species (<i>P. virescens</i>) | 0.389 | 0.136 | 2.871 | 0.004 | Treatment (Mixed) | 1.325 | 0.295 | 4.492 | <.0001 |
| Sex (Male) | -0.153 | 0.134 | -1.138 | 0.255 | Treatment (Native) | 2.358 | 0.194 | 12.165 | <.0001 |

Table 4. Pairwise comparisons between all combinations of significant predictor variables in our statistical analyses. Multiple comparisons are corrected using a Tukey HSD multiplicity adjustment. Significant comparisons are indicated in bold.

| a) Sheltering behaviour | | | | |
|--|---------------|--------------|----------------|-------------------------|
| <i>N_{obs}</i> = 672, <i>N_{ind}</i> = 32 | | | | |
| Contrast | β | SE | z-value | <i>p_{corr}</i> |
| <i>P. sicula</i> , conspecific - <i>P. virescens</i> , conspecific | 0.628 | 0.133 | 4.728 | <.0001 |
| <i>P. sicula</i> , conspecific - <i>P. sicula</i> , heterospecific | 0.192 | 0.192 | 0.999 | 0.75 |
| <i>P. sicula</i> , conspecific - <i>P. virescens</i> , heterospecific | 0.394 | 0.231 | 1.709 | 0.319 |
| <i>P. virescens</i> , conspecific - <i>P. sicula</i> , heterospecific | -0.436 | 0.231 | -1.89 | 0.232 |
| <i>P. virescens</i> , conspecific - <i>P. virescens</i> , heterospecific | -0.234 | 0.193 | -1.211 | 0.62 |
| <i>P. sicula</i> , heterospecific - <i>P. virescens</i> , heterospecific | 0.202 | 0.138 | 1.472 | 0.454 |
| b) Amount of food | | | | |
| <i>N_{obs}</i> = 288, <i>N_{ind}</i> = 32 | | | | |
| Contrast | β | SE | z-value | <i>p_{corr}</i> |
| <i>P. sicula</i> , conspecific - <i>P. virescens</i> , conspecific | 0.26 | 0.176 | 1.479 | 0.45 |
| <i>P. sicula</i> , conspecific - <i>P. sicula</i> , heterospecific | -0.383 | 0.142 | -2.699 | 0.035 |
| <i>P. sicula</i> , conspecific - <i>P. virescens</i> , heterospecific | 0.601 | 0.226 | 2.661 | 0.039 |
| <i>P. virescens</i> , conspecific - <i>P. sicula</i> , heterospecific | -0.643 | 0.209 | -3.073 | 0.011 |
| <i>P. virescens</i> , conspecific - <i>P. virescens</i> , heterospecific | 0.341 | 0.172 | 1.98 | 0.195 |
| <i>P. sicula</i> , heterospecific - <i>P. virescens</i> , heterospecific | 0.984 | 0.202 | 4.862 | <.0001 |
| c) Weight | | | | |
| <i>N_{obs}</i> = 96, <i>N_{ind}</i> = 32 | | | | |
| Contrast | β | SE | z-value | <i>p_{corr}</i> |
| <i>P. sicula</i> , conspecific - <i>P. sicula</i> , heterospecific | -3.812 | 1.189 | -3.205 | 0.007 |
| <i>P. sicula</i> , conspecific - <i>P. virescens</i> , conspecific | -4.381 | 2.763 | -1.586 | 0.387 |
| <i>P. sicula</i> , conspecific - <i>P. virescens</i> heterospecific | -4.547 | 2.847 | -1.597 | 0.380 |
| <i>P. sicula</i> , heterospecific - <i>P. virescens</i> , conspecific | -0.569 | 2.847 | -0.200 | 0.997 |
| <i>P. sicula</i> , heterospecific - <i>P. virescens</i> , heterospecific | -0.735 | 2.928 | -0.251 | 0.994 |
| <i>P. virescens</i> , conspecific - <i>P. virescens</i> heterospecific | -0.166 | 1.189 | -0.140 | 0.999 |
| d) Number of contacts | | | | |
| <i>N_{obs}</i> = 128, <i>N_{ind}</i> = 32 | | | | |
| Contrast | β | SE | z-value | <i>p_{corr}</i> |
| Conspecific Invasive - Heterospecific Mixed | -1.325 | 0.295 | -4.492 | <.0001 |
| Conspecific Invasive - Conspecific Native | -2.358 | 0.194 | -12.165 | <.0001 |
| Heterospecific Mixed - Conspecific Native | -1.032 | 0.278 | -3.719 | 0.001 |

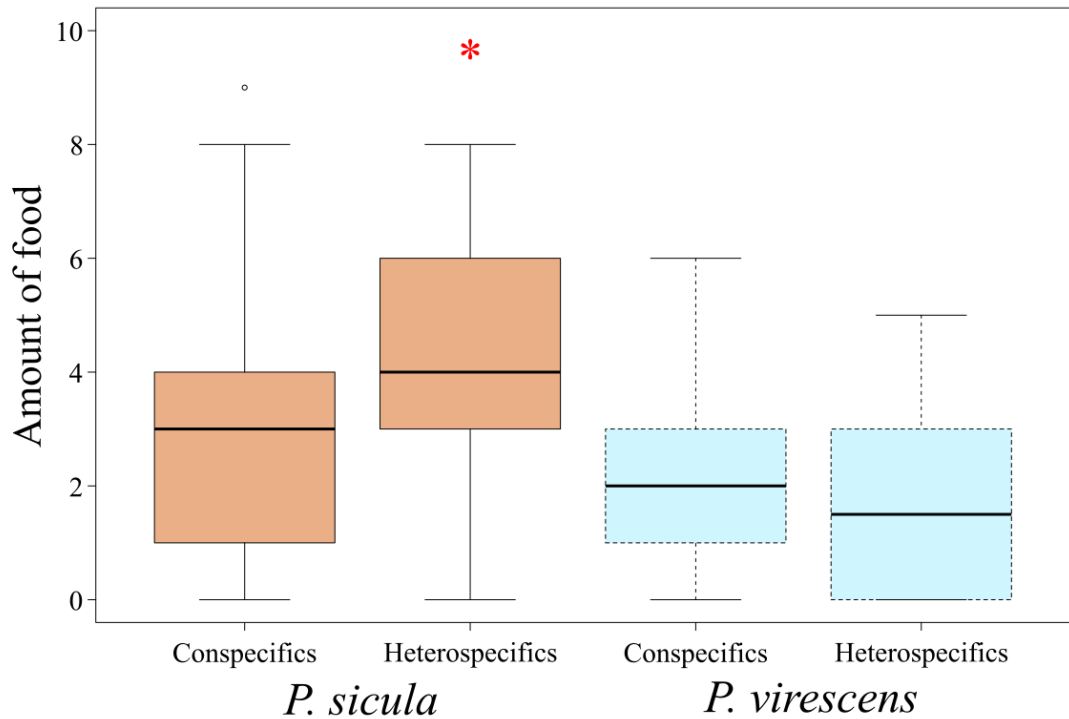


Fig 2. Amount of food (number of mealworms) eaten by each species (*P. sicula* in orange with solid lines, and *P. virescens* in blue with dashed lines) for both treatments (conspecifics and heterospecifics). The red asterisk represents *P. sicula* in the heterospecific treatment, which ate significantly more food than all other treatment and species combinations. This boxplot was plotted from raw data.

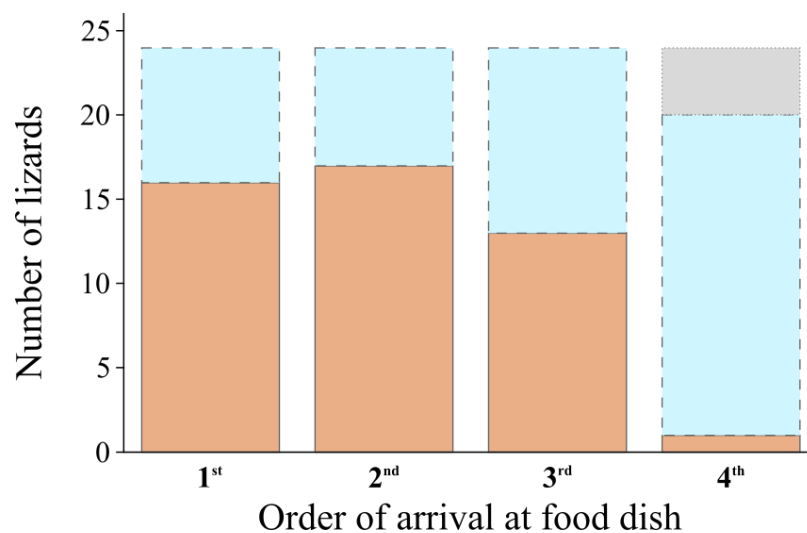


Fig 3. Order by which species would arrive at the food dishes during the heterospecific treatment. *Podarcis sicula* (orange with solid lines) typically arrived before *P. virescens* (blue with dashed lines). The grey area with dotted lines represents the lizards that did not approach the food dishes. Visualisations were from raw data.

Stealing food and aggressive behaviour

The native *P. virescens* were more likely to attempt to steal food than the invasive *P. sicula* during the conspecific treatment (Fig 4a). However, when in the heterospecific treatment, *P. virescens* attempted to steal food much less, either from conspecifics or from heterospecifics (Fig. 4a). In contrast, *P. sicula* attempted to steal food more often when in the heterospecific treatment than when in the conspecific treatment, but these attempts were mostly directly towards conspecifics (Fig 4a). Moreover, the majority of aggressive interactions (biting and fights) we observed between lizards were related to food (Fig 4b). When housed with conspecifics, *P. virescens* exhibited the most aggressive interactions (Fig 4b).

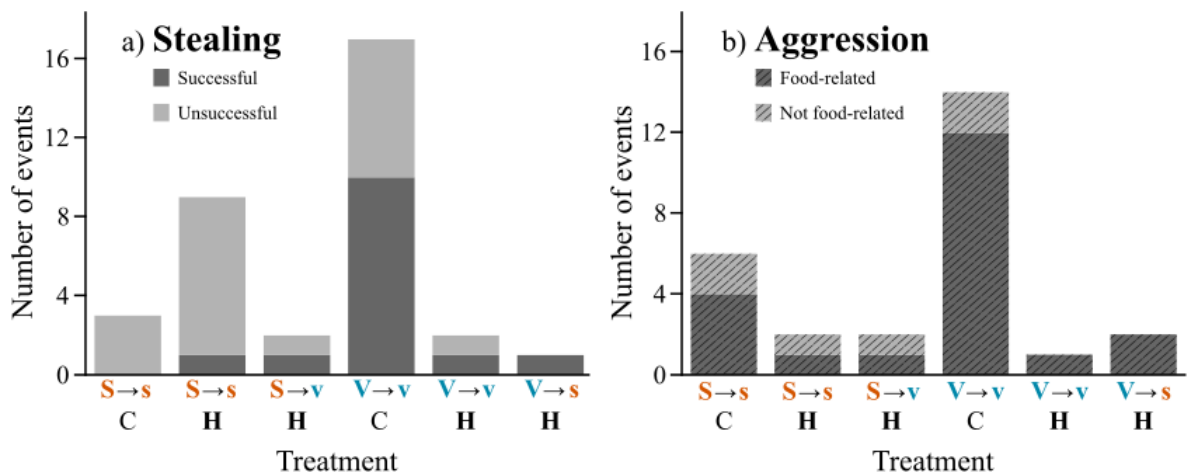


Fig 4. Number of incidents of food-stealing (a), and of aggression (b) scored throughout the entire experiment (continuously from 08:00 – 19:00 h). For each treatment C=conspecifics and H=heterospecific and the arrow indicates the direction of the behaviour (instigator to recipient). *P. sicula* is represented in orange “S” or “s”, and *P. virescens* in blue “V” or “v”. In graph a), dark grey indicates successful food-stealing events, while light grey represents failed stealing events. In graph b), striped dark grey represents aggression related to food, and striped light grey represents aggression unrelated to food.

Social tolerance

In the conspecific treatment, *P. sicula* were observed in contact 399 times. In contrast, *P. virescens* were observed in contact 1207 times. In the heterospecific treatment, lizards were observed in contact 562 times. Of these interactions in the heterospecific treatment, 66 were between *P. sicula*, 169 were between *P. virescens*, and 327 were between heterospecifics. The number of observations of lizards in contact while basking increased as the experiment progressed (Table 3 f). Within the conspecific treatment, *P. virescens* were observed in contact more frequently than *P. sicula* (Table 4 d). Moreover, the overall number of contacts between lizards in the heterospecific treatment was higher than those observed for *P. sicula* with conspecifics, but lower than those observed for *P. virescens* with conspecifics (Table 4 d).

DISCUSSION

We found evidence for exploitative competition between the invasive *P. sicula* and native *P. virescens*. Specifically, when both species were together, there were no differences in their sheltering behaviour, their level of use of the high-quality area, in stealing food, nor in their aggressive behaviours, all of which would be indicative of interference competition. Instead, *P. sicula* arrived first at the food dishes, ate more food, and consequently gained more weight. This evidence is consistent with exploitative competition. We also found evidence of social tolerance (both species would share shelters and the high-quality area, and be in contact with each other). Even though it does not depend on direct interactions, exploitative competition can have a significantly negative impact on a species. For example, the invasive common house gecko *Hemidactylus frenatus* can rapidly displace the native mourning gecko (*Lepidodactylus lugubris*) across the Pacific basin, solely through exploitative competition (Petren 1993, Petren and Case 1996).

Podarcis sicula modified its feeding behaviour in the heterospecific treatment and started to eat more, compared to the conspecific treatment, while also increasing its weight. Becoming larger when with heterospecifics was also a factor during interference competition experiments between *P. sicula* and *P. melisellensis* (Downes and Bauwens 2002). In the heterospecific treatment, the invasive *P. sicula* was first to arrive at the food dishes and consumed more than the native species. In species that live in

groups, being the first to arrive at a food source, and eating a greater quantity of food, is common in dominant individuals (Baker et al 1981, Beauchamp 2013) even if they do not display overtly aggressive behaviour. This pattern has also been documented in the invasive Argentine ant (*Linepithema humile*) and in the common house gecko (*Hemidactylus frenatus*) when they are in competition with native species (Holway 1999, Petren and Case 1996). Invasive species can out-compete native species simply by obtaining more, and sometimes better, food (Holway 1999, Human and Gordon 1996), thereby reducing the levels of available resources (Petren and Case 1996).

Although *P. virescens* did not lose weight in the heterospecific treatment compared to the conspecific treatment, this may be because there were still relatively large quantities of food available. These effects are likely to be more profound in the wild, especially during periods when food is more restricted (such as in summer or in winter because of a limited activity window). Also, an invasive species can dominate invaded areas by establishing high densities, which promotes its increasing foraging voracity (Pintor et al 2009). It is possible that increased densities of *P. sicula* in the wild can have a real impact on the foraging success of the native species and lead to its displacement.

These results can have major implications for understanding the impact of this invader on the native communities. The competitive superiority of exotic species over native species, jointly with the use of ecological opportunities derived from human actions, is a major explanation for the paradox of biological invasions: how can a species with no evolutionary history in a given location, be able to become established, dominant, and even displace native species, when the latter had much more time to adapt to local conditions (Sax et al 2000)? Two arguments can explain this competitive superiority. First, invasive species may have a longer history in human-modified ecosystems and therefore, they may be better adapted to urban environments than native species (Elton 1958). However, Lisbon is an old European city, occupied by humans for millennia, and thus *P. virescens* should not have experienced any less selective pressure from urbanisation than *P. sicula* in its native range in Italy. Second, the novel location might have fewer enemies (such as predators or parasites that are not adapted to interact with the introduced species; Roughgarden 1983). But again, this hypothesis explains little, as both species are closely related and have similar ecological requirements, so should be thus exposed to similar selective pressure, such as predators. On the other hand, one explanation for the ability of *P. sicula* to exploit food resources in the presence of the native species may be due to the invasive

species' boldness (Short and Petren 2008, Damas-Moreira et al *chapter III*). Similarly, *P. virescens* may become shier in the presence of the invasive *P. sicula*. Indeed, the native *P. virescens* reduced their frequency of interactions from the conspecific to heterospecific treatment (becoming less aggressive, less socially tolerant, and attempting to steal food less), and they were the last to arrive at the food source. This suggests that *P. virescens* changed its social interactions in the presence of *P. sicula* and may avoid foraging at the same time as the invasive species. A similar behavioural avoidance during foraging is at play in native geckos (Petren et al 1993), and this may explain why *P. sicula* is not directly aggressive towards the native *P. virescens*. Also, in Sardinia, the introduced *P. sicula* and the native Tyrrhenian wall lizard *Podarcis tiliguerta* sometimes share microhabitats, but the latter species becomes more cautious when *P. sicula* are present. When *P. tiliguerta* avoids aggressive encounters with *P. sicula*, one possibility is that it might result in later habitat segregation (Vanhooydonck et al 2000). The behavioural responses from the native species can be key to understanding the type of competition used by *P. sicula*, and also help explain its success as an invasive species (Strauss et al 2006, Langkilde et al 2017). For example, the native skink *Caledoniscincus austrocaledonicus* is able to avoid unknown competitors without prior contact, which might help reduce the impact or frequency of costly aggressive encounters between the two species if they come into contact (Gérard et al 2016). Simultaneously, this can facilitate the establishment of an invader, by having reduced competition obstacles.

While we found evidence for exploitation competition, Downes and Bauwens (2002) documented interference competition for a population of *P. sicula* in Croatia. This suggests a degree of plasticity in which *P. sicula* may adopt a different strategy depending on the context and potentially, the intrinsic properties of the native species. A similar pattern has been documented in squirrels; the invasive grey squirrel has largely displaced red squirrels in Europe, but the type of competition they employ is not the same across all invaded areas (Wauters et al 2001, Gurnell et al 2004). Switches between exploitative and interference competition may be in response to resource availability and population density (Holdridge et al 2016). In our study, the strategy of exploitative competition may be in response to the behaviour of the other species, the density of lizards, food and resource availability, or some interaction of all of the above. Direct interactions can be costly to both parties because they carry an energetic cost, are stressful, and they risk injury. If the costs of interference competition are

sufficiently high, the ability to alter behaviour in a manner that favours exploitative competition is an alternative with a potentially high pay off. We suggest that invasive species are a good model with which to test predictions related to competition and the degree to which a species can adjust its behaviour. A species that can plastically adjust its competition strategy to the surrounding environment and displace sympatric native species is likely to be a successful invader.

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ETHICS: Research approved by the Macquarie University Animal Ethics Committee (ARA2017/004) and by the Portuguese Institute for Conservation of Nature and Forests (ICNF) (License 428/2017/CAPT).

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ELECTRONIC SUPPLEMENTARY MATERIAL

Damas-Moreira I, Riley JL, Carretero MA, Harris DJ, Whiting MJ. 2018. Getting ahead:

Exploitative competition by an invasive lizard

Experimental protocol

Lizards were allocated in the tubs accordingly to their SVL (summary of SVLs in Table S1). A timeline with a distribution of the lizards throughout the treatments can be found in Fig S1.

Table S1. The mean difference in snout-to-vent length (SVL) between lizards allocated to different treatments (mm). The standard deviation (SE) follow all means.

| | | | SVL \pm SE |
|--------------------------|---------------------|---------------------|------------------|
| Conspecific treatment | <i>P. sicula</i> | Between males | 1.05 \pm 0.31 |
| | | Between females | 0.95 \pm 0.27 |
| | | Between all lizards | 9.47 \pm 0.51 |
| | <i>P. virescens</i> | Between males | 4.67 \pm 0.80 |
| | | Between females | 3.00 \pm 0.44 |
| | | Between all lizards | 5.63 \pm 0.47 |
| Heterospecific treatment | Between species | Between males | 16.45 \pm 0.69 |
| | | Between females | 9.77 \pm 0.49 |
| | | Between all lizards | 18.25 \pm 0.33 |

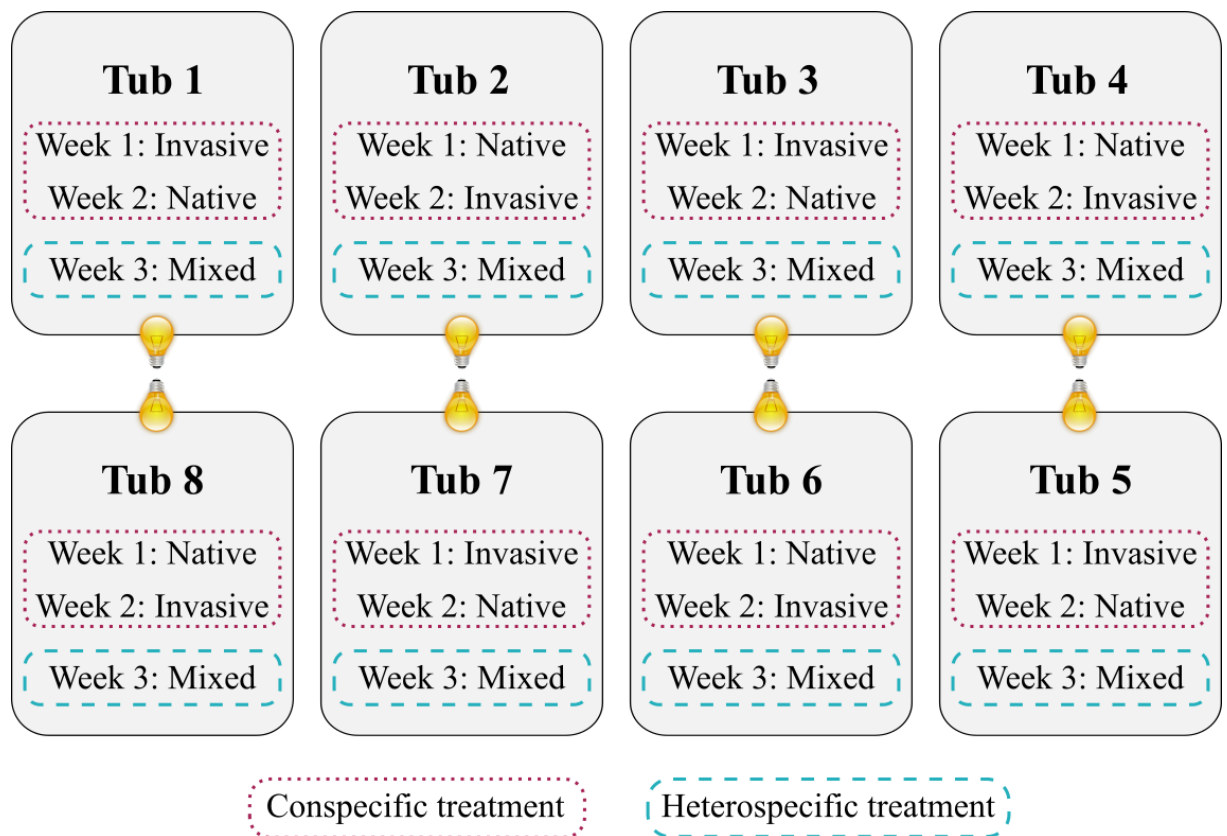


Fig S1. Schematic representation of the distribution of treatments and species across the three experimental weeks. The groups we used in the conspecific treatment in the analysis is within dotted lines and the group we used in the Heterospecific treatment is within dashed lines. The number of lizards inside each tub was always 2 males and 2 females ($n=4$), in every week and treatment.

GENERAL DISCUSSION

The chapters in this thesis give exciting insights into the invasive mechanisms of the Italian wall lizard, *Podarcis sicula*, and contribute to our understanding of the behaviour of invasive species. Briefly, in order to analyse the behavioural mechanisms that can make a species successful during an unintentional invasion process, I studied the role of cognition and behaviour in a population of *P. sicula*. In particular, I addressed their behavioural flexibility (chapter I), use of social information (chapter II), behavioural traits linked to invasion success (chapter III), and its competitive ability with a native congeneric species (chapter IV).

Implications for biological invasions

The Italian wall lizard had different cognitive abilities than non-invasive congeneric species, and relatively greater levels of behavioural flexibility (measured by reversal learning ability, chapter I). Also, it was able to use social information to solve a novel task, learning equally from both conspecifics and heterospecifics (chapter II). When compared with a sympatric native *Podarcis* species, I found that behavioural traits generally linked to invasion success, greatly differed between the two species (chapter III). The native species was less exploratory, bold, and more neophobic, and more repeatable in its behaviour than *P. sicula* (chapter III). Conversely, the invasive species was less consistent (only repeatable for boldness) and the behavioural traits were not correlated (chapter III). Lastly, to understand the mechanism underlining the competitive advantage over native species, I examined their behaviour when the two species were in direct contact (chapter IV). The invasive species did not aggressively compete with the native species, instead was socially tolerant, but nonetheless consumed more resources (chapter IV). This result was consistent with exploitative competition (chapter IV).

The research within this thesis revealed that it is thus likely that *P. sicula* is a species with plastic behaviours and moderate behavioural flexibility (chapter III and I), and able to use social learning from the same or different lizard species to quickly adapt to novel situations (chapter II). Particular

behavioural traits can also give them an advantage over native species. By being more exploratory, bold, and neophilic (chapter III), it can arrive and obtain more food by out-competing native species through exploitative competition (chapter IV). All of this can help explain why *P. sicula* is such a successful invader and which mechanisms it uses to thrive in new locations, whereby they gain an advantage over native species.

In particular, I would like to highlight and discuss my three favourite findings from this thesis.

Not repeatable and not correlated: The Italian wall lizard was much more exploratory, bold, and neophilic than a native congeneric sympatric species (chapter III). Being bolder, more exploratory, and neophilic might enhance the success of invaders during the invasion process beginning with the point where it needs to enter a transport vector, to when it arrives and settles in a new location, until the point where it becomes established and thrives (Chapple et al. 2012, Griffin et al. 2016, Rehage and Sih 2004, Short and Petren 2008). The findings in chapter III also showed that while the native resident *P. virescens* was highly consistent in its behaviours and all three traits were correlated (the more exploratory individuals were also those bolder and more neophilic), the invasive species was only repeatable for boldness, and lacked a correlation in behavioural traits (chapter III). This can indicate a greater degree of plasticity in the Italian wall lizard. The hypothesis that plasticity should aid animals to invade novel environments is not a new idea (Mayr 1965) and, given that during the entire invasion process animals may be constantly dealing with new challenges (Blackburn et al 2011), having plastic behaviours, instead of having a fixed personality type, may be of major importance. This is because plasticity can increase the probability of the existence of adaptive behaviours to different situations, enhancing the progress of the individual and population during the invasive process (Dingemanse and Wolf 2013, Sih et al 2012). Although several studies found correlated behavioural traits to promote invasive species success (invasion syndromes; e.g. Cote et al. 2010, Michelangeli et al. 2017, Rehage and Sih 2004), not presenting this correlation can likely allow a population to adapt with more efficiency. This is because correlated behaviours can constrain the population in the sense that if selection acts on one behaviour, it can affect correlated ones (Sih et al. 2012). However, the relationship between personality and individual plasticity is complex and they are

not mutually (Dingemanse et al 2010). In mice, less aggressive individuals regulate their level of aggression depending on social context, but more aggressive individuals do not (Natarajan et al 2009). The context of an invasion can thus be important but, overall, having individuals in a population exhibiting different behaviours, and not always behaving in the same way, can give a species the necessary edge during all stages of the invasion process.

The role of cognition: Chapter I and II joins previous studies (see Weis and Sol 2016) in the suggestion that cognition may play an important role during invasions. In particular, chapter II shows the first evidence of learning from different species (they never encountered before in nature) to solve a novel task, in the same way as learning from a conspecific, within the context of an invasion. Such an ability could provide a significant advantage to an invasive species, as being able to learn from a different (native) species could allow an animal to learn where to find food sources or suitable shelters as soon as they arrive at a novel location, thereby avoiding the risk of investigating, possibly risky, territory. Plausibly, this ability of taking advantage of the behaviour of native residents can facilitate the establishment of newly introduced species, and points to the potentially important role of native species in determining the success of an invasion. For example, due to human-related conflicts, populations of African elephants (*Loxodonta africana*) often need to be purposely relocated. One aspect of a successful translocation, is that the population that is able to survive in the novel environment gradually modifies its behaviour (time spent foraging, walking, and standing) to more similar to local resident elephants (Pinter-Wollman et al 2009). In a parallel way, black rats (*Rattus rattus*) can invade a new forest by learning a new specific foraging technique from other individuals (Terkel 1995). *Podarcis* lizards are considered less social animals although they may occasionally aggregate in the presence of food (*Podarcis lilfordi*, Pérez-Cembranos and Pérez-Mellado 2014) or overnight in a common refuge (*Podarcis muralis*, Aubret et al 2014). But social learning can be equally relevant for less social species in diverse taxa, such as in octopuses (*Octopus vulgaris*, Fiorito and Scotto 1992), tortoises (Wilkinson et al 2010), or fishes (*Spinachia spinachia*, *Cottus gobio*, *Barbatula barbatula*, and *Platichthys flesus*, Webster and Laland 2017). The fact that social learning occurs outside social species can be because all animals at some point in their life are exposed to social information, from conspecifics or heterospecifics that share resources or threats (Avarguès-

Weber et al 2013, Coolen et al 2003, Goodale et al 2010, Seppänen et al 2007, Webster and Laland 2017). Despite all of this, and with a large body of evidence pointing to social learning as a rapid transfer of knowledge at low cost (Hoppe and Laland 2008), the use of public information as an advantage during biological invasions has received little attention. I hope the findings in chapter II will act as a catalyst for future studies addressing the role of social learning during the invasive process.

Native species response: The way in which a native species responds to an introduced species can help determine both the success of the native species in dealing with this new invader, and the success of the invasion (Langkilde et al 2017, Strauss et al 2006). Native species often need to alter their behaviour so they can cope with an unknown species. In New Zealand, the invasive brown trout (*Salmo trutta*) replaced the native common river galaxias (*Galaxias vulgaris*); because the introduced trout is diurnal but the native galaxias is nocturnal, this caused their common prey, the mayfly (*Nesameletus ornatus*) to shift its activity period from diurnal to nocturnal in invaded river streams to avoid the new predator, which in turn can have consequences in the stream community (McIntosh and Townsend 1994). In another instance, the red imported fire ant (*Solenopsis invicta*) can strongly affect invaded ecosystems and arthropod communities (Holway et al 2002), and even vertebrates will modify their behaviour in response to their presence (Ligon et al 2012). For example, the eastern bluebird (*Sialia sialis*) shifts its foraging behaviour (foraging farther away from their nests) and rate of provisioning (provisioning less) in response to the presence of this devastating invader (Ligon et al 2012).

During the competition experiment (chapter IV), I also found a behavioural response from the native *P. virescens* to the presence of the invasive *P. sicula*. In this experiment, both native and invasive species were maintained with only conspecifics before being introduced with heterospecifics. This was to establish if there were behavioural changes in the presence of a heterospecific and if one species would dominate the other. Interestingly, *P. virescens* had more aggressive behaviours (biting, fights, and stealing food) when held with conspecifics, than when the invasive species was present. Also, when both species were together, the invasive species would mainly arrive at the food dish before the native species. This may suggest a level of wariness on the part of the native species (chapter IV). In a parallel study, a native species, *Podarcis tiliguerta*, when in the presence of *P. sicula* shows a level of

avoidance and caution (Vanhooydonck et al 2000). This avoidance also happens with the native mourning gecko when in the presence of the invasive common house gecko (Petren et al 1993) or in the Pacific mud snail (*Cerithidea californica*) in the presence of the invasive Atlantic snail (*Ilyanassa obsoleta*; Race 1982). These studies, and the results in this thesis, illustrate the importance of what species are on site during an invasion because their reaction to an invader could influence the probability of an invasion being successful or unsuccessful. Even the invasive success of plant species can depend on the behaviour of native animal species. Pollinators, such as bees and beetles, can distinguish between native and alien plants and exploit more one of the two options, shaping the invasive success of the exotic plant (Bartomeus et al 2008). The behavioural responses from native species may in part explain the success of *P. sicula* and of many other different invaders.

Conservation

Although an early intervention can prevent the establishment of *P. sicula* (Hodgkins et al 2012), it is now probably too late for a successful eradication of this species in Lisbon. In several case studies of reptile invasions, management actions (measures or quantifications since the introduction, or eradication attempts) are practically non-existent during the early stage of introduction (Kraus 2009). This also happened in the case of the *P. sicula* population in Lisbon; my research and previous studies indicate that any delay in actions might constitute a future problem, as there will be little hope of preventing the spread of the species.

Podarcis sicula have higher levels of exploration, boldness, and neophilia (chapter III), and forages more efficiently than sympatric native species (chapter IV). It is therefore likely that it will only continue to expand its range. These behaviours can increase the likelihood that lizards will actively find and enter nearby cargo (Sih et al 2004). Moreover, although this species is not consistent in their exploratory and neophilic behaviour, *P. sicula* is consistently bolder (chapter III). This trait can be of major importance during an invasion. Boldness can influence if an animal is transported outside of its native range, and, after introduction in a novel location, bolder individuals may have greater access to resources (Monceau et al 2015, Pintor et al 2008, Short and Petren 2008). However, studies conducted in Australia and New Zealand suggest that being bold can also be risky for invaders during transportation and colonization (Chapple et al 2011, Gill et al 2001, Kraus 2009), because it can

increase their detection and, in turn, their chances of being caught or eliminated (Chapple et al 2012). Nevertheless, Australia and New Zealand are both countries where laws regarding the unintentional transportation of non-native species are stricter than those within the Mediterranean Basin and North America. For example, in the Mediterranean and across Europe, preventive actions against the movement of invasive species are minimal to non-existent, and those that do exist focus mainly on species that could impact agriculture or fisheries (Genovesi et al 2015, Hulme 2007). Therefore, the constraints of being bold, that have been found in Australia and New Zealand, may not apply to *P. sicula* and, in contrast, it appears that boldness enhances invasion success in this species. Moreover, boldness can confer an advantage in urban environments because it translates into greater foraging efficiency (Short and Petren 2008), which I documented in chapter IV.

The high urbanisation levels in Parque das Nações are thought to constrain the dispersal of *P. sicula* (through the presence of many artificial structures and constant garden maintenance; Ribeiro et al 2017). Nonetheless, these lizards live in an environment particularly amenable to hitchhiking to new locations. This site has constant activity, with lots of public and private transportation, or cargo trucks. For example, the closest marina and wharf is only less than 2 km away. The population also lives less than 10 km away from all sorts of long-range cargo transportation: truck transport companies, maritime ports, docks, and wharfs (that connect to numerous other countries). Allied to their behavioural mechanisms (chapter I, II, III, IV), this opportunistic location can help *P. sicula* to expand its range within Lisbon and beyond (Carretero and Silva-Rocha 2015). Interestingly, another invasive lizard, the Madeira lizard, *Teira dugesii*, arrived from the Portuguese island Madeira through maritime cargo transportation to Lisbon (Silva-Rocha et al 2016), and now lives only 5 km away from *P. sicula*. This raises the question: what prevents the Italian wall lizard from travelling the inverse route and reaching and establishing in Madeira (Carretero and Silva-Rocha 2015)? In fact, there is a general concern that this population from Lisbon will be able to reach neighbouring islands such as Madeira, the Azores, or Canary islands (CABI 2018), all of which have direct connections to Lisbon.

Given all of this, conservation efforts should continuously monitor the rate and pattern of the spread of *P. sicula*, as well as changes in the distribution of the native *P. virescens* (Simberloff et al 2013, Lewis et al 2016). Moreover, introduced species can also effect native prey populations (Pintor et al 2009); we do not know if the greater foraging efficiency of *P. sicula* (chapter IV) can also impact native

invertebrate communities. Changes in native invertebrate communities can also impact native species that feed on the same resources. For example, during field work I observed *Psammodromus algirus* and *Tarentola mauritanica* also inhabiting the same area in Parque das Nações as *P. sicula*.

Psammodromus algirus makes use of different habitat types and it was living less than 200 meters away from *P. sicula*, and *T. mauritanica* was found in syntopy with *P. sicula* (probably because they have opposite activity periods, as *T. mauritanica* is crepuscular and nocturnal). Both these lizards (and *P. virescens*) have similar diets to *P. sicula*, and plausibly, could be affected through declining resource availability.

Lastly, invasive species can have other ecological impacts that are less well understood. Besides the direct impact on native lizards and prey, the effect of novel parasites should always also be taken into account. Parasites that are passively carried with introduced species are seldom studied but can have a significant impact on invaded communities (Poulin 2017). In the case of *P. sicula*, it might be that there are no significant impacts from parasites on novel locations because they are likely not carrying many or losing them after introduction (appendix I, Burke et al 2007). During my research, I was able to verify that the population in this study site had almost no blood parasite infections (appendix I). Similarly, invasive *P. sicula* populations in North America also host only small numbers of blood and intestinal parasites (Burke et al 2007). It seems that blood parasites are not a matter of concern during the invasion of *P. sicula*, but instead another probable advantage for the hosts during the invasive process.

Conclusions

This research contributes to our knowledge about the role behaviour can play in the invasion process. It adds evidence that flexibility can be key to deal with unpredictable environments and situations (chapter I and III), and highlights the role native species can play in the success of non-native species (chapter II and IV). Overall, all results in this thesis also converge to one major point: behaviour is likely a crucial mechanism underlining biological invasions, and can be part of determining if a species becomes a successful invader or not. This is supported by my results and by a large body of evidence that suggests the same importance of behaviour in other invasive cases (reviews in Holway

and Suarez 1999, Chapple et al 2012, Weis and Sol 2016). As Suarez and Cassey (2016) stated, “behaviour is the mechanistic link that determines how species interact with each other and their environment and is therefore essential for understanding why some species succeed in new environments and what impacts they will have once established”.

As unintentional translocations and the establishment of alien species will not slow in the near future (Seebens et al 2017), I believe future research should incorporate behaviour and cognition for a fuller understanding of what make some species prone to become successful invaders and to develop conservation strategies. Because I agree there is not one unique independent reason to be a successful invader (Sol and Maspons 2016), I used an approach in which I performed different experimental assays to allow a more complete characterization of the Italian wall lizards’ behaviour. Future approaches should greatly benefit from incorporating different behavioural aspects, as well as other potentially important features such as life history, physiology, endocrinology, environmental conditions, or urbanisation, in order to unravel how all of these traits correlate and how they impact the invasion process. More research should also involve behavioural components that may give an immediate edge to invasive species during establishment, as this should be the primary advantage for invaders that hitchhike on transport, to successfully establish and spread. As my findings suggest, a fruitful approach to this end can be to compare closely related species with different invasive potential.

Research on the role of behaviour during invasions has already demonstrated the profound effect behaviour can have on the success of a species in novel environments (reviewed in Chapple et al 2012, Weis and Sol 2016). Future research will hopefully bring more promising and exciting results. I believe it will help us better understand what promotes the success of an invasive species, and that such research will be able to strongly enrich conservation efforts.

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APPENDICES

Appendix I

Paper related to this PhD research

Damas-Moreira I*, Tomé B*, Perera A, and Harris DJ. *Can blood parasites mediate the success of an invasive lizard?* In preparation for submission.

* Both as first authors

Abstract

During biological invasions, invaders can carry other species along with them, an occurrence often neglected. Parasites are one such clandestine passenger. For instance, blood parasites live inside their hosts and, as such, they are passively translocated wherever hosts go. Although they are discrete organisms, parasites are important pieces of the ecosystem; they influence their hosts' fitness and interactions, and can thus shape the distribution of biodiversity and the ecological structure of an environment. A better picture of their role in introductions can be thus essential to fully understand the invasion potential of their hosts. Using a population of the invasive Italian wall lizard *Podarcis sicula*, we compared the infection levels of haemogregarines, a common blood parasite of reptiles, with the sympatric congeneric native green Iberian wall lizard *Podarcis virescens* in Lisbon, Portugal. By screening a total of 29 *P. virescens* and 27 *P. sicula* males, we found great differences in the prevalence of haemogregarine parasites. While *P. virescens* lizards were frequently infected (69.0%), only one individual of *P. sicula* was found to be infected (3.7%). Moreover, *P. virescens* exhibited an average of 1.36% of infected erythrocytes, against only 0.04% in the one *P. sicula* individual. Genetic analyses confirmed these blood parasites to be haemogregarines, of two of the various haplotypes that commonly infects Iberian *Podarcis* species. Unfortunately, due to the low levels of infection, we were not able to amplify parasite DNA from the infected *P. sicula* individual (although it is morphologically similar to one of the haplotypes found in the native lizards). Low levels of parasites are a common thread in *P. sicula*: it has been shown in its native range, as well as in introduced populations in the USA. *Podarcis sicula* is a highly adaptable lizard, with introduced populations in three continents, and haemogregarine parasites might lower the competitiveness of their hosts. As so, we suggest that this general lack of parasites can provide a competitive advantage over native lizard species, and help understand the great invasive potential of *P. sicula*.

Appendix II

Official presentations given about the research from this thesis

16th Congress of the International Society for Behavioral Ecology

Exeter, England

July, 2016 - Poster presentation

Damas-Moreira I, Harris DJ, and Whiting MJ

Do invasive lizards conquer through learning?

Behaviour 2017, World congress

Estoril, Portugal

August, 2017 - Oral communication + volunteering during the congress

Damas-Moreira I, Ana Pereira, Harris DJ, and Whiting MJ

Behaviour flexibility in an invasive lizard

Australasian Evolution Society Conference

Hobart, Tasmania, Australia

December, 2017 - Oral communication

Damas-Moreira I, Pereira A, Oliveira D, Santos JL, Riley JL, Harris DJ, Whiting MJ

Learning Skills in an Invasive Lizard

XV Iberian Congress of Herpetology

Salamanca, Spain

September, 2018 - Oral communication

Damas-Moreira I, Riley JL, Harris DJ, Whiting MJ

An invasive lizard with strong personality?

Appendix III

Articles published during this candidature unrelated to the thesis

Marshall KLA, Philpot KE, Damas-Moreira I, and Stevens M (2015) *Intraspecific colour variation among lizards in distinct island environments enhances local camouflage*. PLoS one, 10(9), e0135241.

Abstract

Within-species colour variation is widespread among animals. Understanding how this arises can elucidate evolutionary mechanisms, such as those underlying reproductive isolation and speciation. Here, we investigated whether five island populations of Aegean wall lizards (*Podarcis erhardii*) have more effective camouflage against their own (local) island substrates than against other (non-local) island substrates to avian predators, and whether this was linked to island differences in substrate appearance. We also investigated whether degree of local substrate matching varied among island populations and between sexes. In most populations, both sexes were better matched against local backgrounds than against non-local backgrounds, particularly in terms of luminance (perceived lightness), which usually occurred when local and non-local backgrounds were different in appearance. This was found even between island populations that historically had a land connection and in populations that have been isolated relatively recently, suggesting that isolation in these distinct island environments has been sufficient to cause enhanced local background matching, sometimes on a rapid evolutionary time-scale. However, heightened local matching was poorer in populations inhabiting more variable and unstable environments with a prolonged history of volcanic activity. Overall, these results show that lizard coloration is tuned to provide camouflage in local environments, either due to genetic adaptation or changes during development. Yet, the occurrence and extent of selection for local matching may depend on specific conditions associated with local ecology and biogeographic history. These results emphasize how anti-predator adaptations to different environments can drive divergence within a species, which may contribute to reproductive isolation among populations and lead to ecological speciation.

Rosado D, Harris DJ, Perera A, Jorge F, Tomé B, Damas-Moreira I, Tavares I, Estrela H, de Sousa A, Pereira A, Mantovani M, and Salvi D (2016) *Moroccan herpetofauna distribution updates including a DNA barcoding approach*. Herpetozoa, 28 (3/4).

Summary

This paper gathers further sample points of reptiles and amphibians throughout Morocco. In total, 138 localities were sampled and 53 species recorded. Additionally, in cases where a species diagnosis based on morphological characters could not be made with certainty, a DNA barcoding approach was used.

Appendix IV

Animal ethics approvals

ARA 2015/038



MACQUARIE
University

ANIMAL RESEARCH AUTHORITY (ARA)

AEC Reference No.: 2015/038-5

Date of Expiry: 31 December 2017

Full Approval Duration: 08 February 2016 to 31 December 2017 (22 months)

This ARA remains in force until the Date of Expiry (unless suspended, cancelled or surrendered) and will only be renewed upon receipt of a satisfactory Progress Report before expiry (see Approval email for submission details).

Principal Investigator:

Dr Martin Whiting
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Associate Investigator:

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Daniel Oliveira +35 1914292927
Joana Rodrigues Lopes dos Santos +35 1916367718

In case of emergency, please contact:
the Principal Investigator / Associate Investigator named above
or the Animal Welfare Officer - 9850 7758 / 0439 497 383,

The above-named are authorised by MACQUARIE UNIVERSITY ANIMAL ETHICS COMMITTEE to conduct the following research:

Title of the project: The Role of Behaviour in Biological Invasions

Purpose: 4 - Research: Human or Animal Biology

Aims: to examine the role of behaviour and if it can confer an advantage during biological invasions, using *Podarcis* lizards as a model.

Surgical Procedures category: 1 - Observation Involving Minor Interference

All procedures must be performed as per the AEC-approved protocol, unless stated otherwise by the AEC and/or AWO.

Maximum numbers approved (for the Full Approval Duration):

| Species | Strain | Age/Weight/Sex | Total | Supplier/Source |
|--------------|----------------------------|------------------|------------|-----------------|
| 27 - Lizards | <i>Podarcis Sicula</i> | Adult/Any/Female | 65 | Wild |
| 27 - Lizards | <i>Podarcis Sicula</i> | Adult/Any/Male | 65 | Wild |
| 27 - Lizards | <i>Podarcis bocagei</i> | Adult/Any/Female | 65 | Wild |
| 27 - Lizards | <i>Podarcis bocagei</i> | Adult/Any/Male | 65 | Wild |
| 27 - Lizards | <i>Podarcis carbonelli</i> | Adult/Any/Male | 60 | Wild |
| 27 - Lizards | <i>Podarcis carbonelli</i> | Adult/Any/Female | 60 | Wild |
| | | | 380 | |

Location of research:

| Location | Full street address |
|---|--|
| CIBIO - Centro de Investigação em Biodiversidade e Recursos Genéticos | Universidade do Porto, Campus Agrário de Vairão, Rua Padre Armando Quintas, nº 74485-661 Porto |

Amendments approved by the AEC since initial approval:

- Amendment #1** - Addition of a new behavioural experiment (Approved by AEC 18 February 2016).
- Amendment #2(a)** - Addition of a new species to include the *Podarcis carbonelli* (Approved by AEC 20 October 2016).
- Amendment #2(b)** - Modification of the protocol (Approved by AEC 20 October 2016).
- Amendment #3** - Additional 80 *Podarcis sicula* (40 males and 40 females) and 40 *Podarcis bocagei* (40 males and 40 females) (Approved by AEC 16 March 2017).
- Amendment #4** - Addition of Ana Isabel Antunes Pereira as Volunteer (Executive Approved. Ratified by AEC 16 Feb 2017).
- Amendment #5** - Addition of Joana Rodrigues Lopes dos Santos as Volunteer (Approved by AEC 16 Feb 2017).
- Amendment #6** - Addition of Daniel Oliveira as Volunteer (Approved by AEC 16 Feb 2017).
- Amendment #7** - Amend fate of animals to humanely euthanize them at the end of the study (Approved by AEC 16 Feb 2017).

Conditions of Approval: N/A

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

Assoc. Professor Jennifer Cornish (Chair, Animal Ethics Committee)

Approval Date: 28 March 2017

ARA 2017/004



MACQUARIE
University

ANIMAL RESEARCH AUTHORITY (ARA)

AEC Reference No.: 2017/004-5

Date of Expiry:

31 March 2018

Full Approval Duration: 01 April 2017 to 30 June 2018 (14 Months)

This ARA remains in force until the Date of Expiry (unless suspended, cancelled or surrendered) and will only be renewed upon receipt of a satisfactory Progress Report before expiry (see Approval email for submission details).

Principal Investigator:

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David Harris: +351 965 897 686

In case of emergency, please contact:
the Principal Investigator / Associate Investigator named above or
Animal Welfare Officer - 9850 7758 / 0439 497 383

The above-named are authorised by MACQUARIE UNIVERSITY ANIMAL ETHICS COMMITTEE to conduct the following research:

Title of the project: Competition and behaviour in biological invasions

Purpose: 4 - Research: Human or Animal Biology

Aims: To examine the role of behaviour and if it can confer an advantage during biological invasions, using *Podarcis sicula* as a model.

Surgical Procedures category: 1 - Observation Involving Minor Interference

All procedures must be performed as per the AEC-approved protocol, unless stated otherwise by the AEC and/or AWO.

Maximum numbers approved (for the Full Approval Duration):

| Species | Strain | Age/Weight/Sex | Total | Supplier/Source |
|--------------|---|-----------------|-------|-----------------|
| 27 - Lizards | Italian wall lizard - <i>Podarcis sicula</i> | Adults/Any/Any | 384 | Wild caught |
| 27 - Lizards | Italian wall lizard - <i>Podarcis sicula</i> | Adults/Any/Male | 50 | Wild caught |
| 27 - Lizards | Green Iberian wall lizard - <i>Podarcis virescens</i> | Adults/Any/Male | 64 | Wild caught |
| 27 - Lizards | Green Iberian wall lizard - <i>Podarcis virescens</i> | Adults/Any/Any | 50 | Wild caught |
| | | | 548 | |

Location of research:

| Location | Full street address |
|--|---|
| CIBIO, University of Porto | Praça de Gomes Teixeira, 4099-002 Porto, Portugal |
| Faculty of Science, University of Lisbon | Rua Ernesto de Vasconcelos, 1749-016 Lisboa, Portugal |

Amendments approved by the AEC since initial approval:

1. Amendment #1 - Change location and type of housing from outdoors at the University of Lisbon to indoors at CIBIO, University of Porto (Approved by AEC 12/04/2017).
2. Amendment #2(a) - Change location of research from Croatia and Athens to CIBIO, University of Porto (Approved by AEC 22/06/2017).
3. Amendment #2(b) - Additional 50 male invasive *P. sicula* and additional 50 male native *P. virescens* (Approved by AEC 22/06/2017).
4. Amendment #2(c) - Additional behavioural experiment to include aggression assay (Approved by AEC 22/06/2017).
5. Amendment #2(d) - Additional procedure to include the removal of a small section of tissue from the tail tip (Approved by AEC 22/06/2017).
6. Amendment #3 - Add David Harris as Adjunct Supervisor (Executive approved. Ratified by AEC 19 October 2017).

Conditions of Approval: N/A

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

A/Prof. Nathan Hart
(Chair, Animal Ethics Committee)

Approval Date: 19 October 2017

Eis aqui (...) onde a terra se acaba e o mar começa.

Behold (...) where the land ends and the ocean starts.

Luís de Camões, *in* Os Lusíadas, 1572.