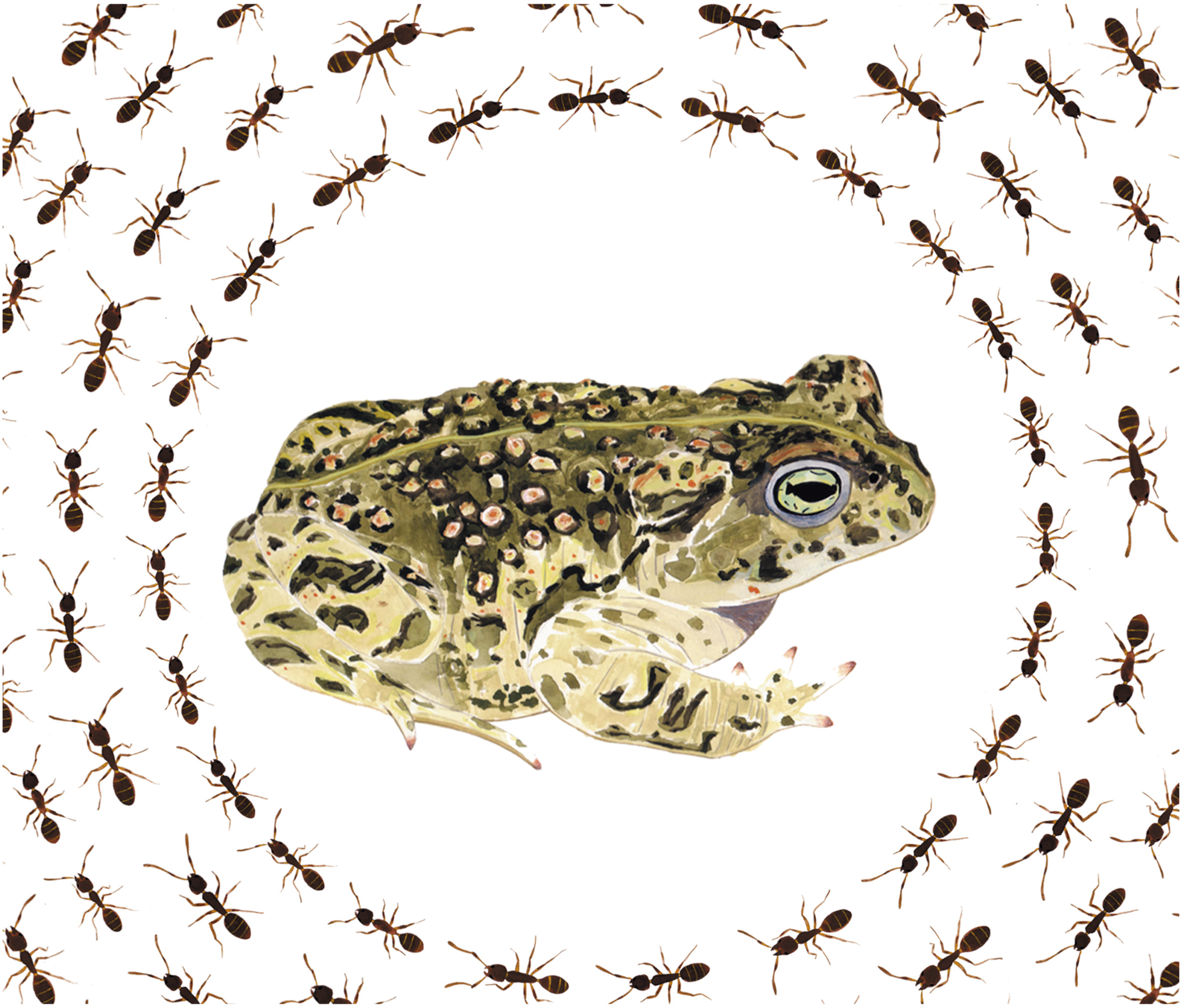
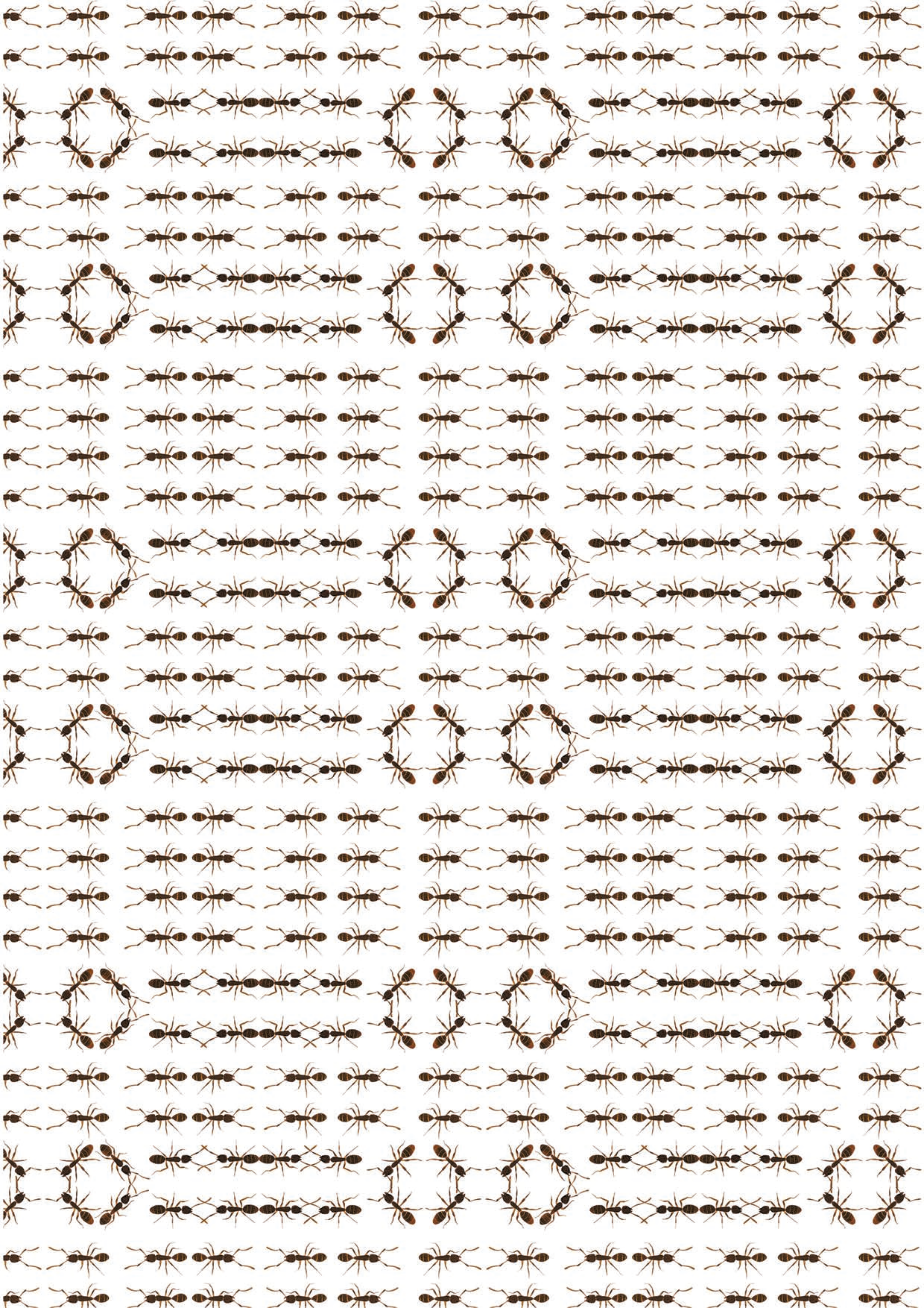
Ecosystem responses to the Argentine ant invasion: Effects on vertebrates



PhD thesis

Paloma Alvarez Blanco



ECOSYSTEM RESPONSES TO THE ARGENTINE ANT INVASION:

Effects on vertebrates

RECOMMENDED CITATION:

Alvarez-Blanco, Paloma (2019). Ecosystem responses to the Argentine ant invasion: Effects on vertebrates. PhD Thesis. Pablo de Olavide University, Seville, Spain.

Front cover and back cover illustrations: María León.

UNIVERSIDAD PABLO DE OLAVIDE
SEVILLA

Estación Biológica Doñana
CSIC
DEPARTMENT of ETHOLOGY
and BIODIVERSITY CONSERVATION

Ecosystem responses to the Argentine ant invasion: Effects on vertebrates

Respuestas del ecosistema ante la invasión de la hormiga argentina: Efectos en vertebrados

Paloma Alvarez Blanco

Supervisors:
Dr. Xim Cerdá Sureda
Dra. Elena Angulo Aguado

Thesis for the degree of Doctor of Philosophy
Universidad Pablo de Olavide
Sevilla, 2019

Dr. Xim Cerdá Sureda
Dra. Elena Angulo Aguado
Paloma Alvarez Blanco

Lo esencial es invisible a los ojos

Antoine de Saint-Exupéry

A mis padres

Table of contents

Abstract

Biological invasions are one of the main drivers of biodiversity loss. Certain effects of invasive species are commonly overlooked and potentially compromise the structure and function of ecosystems in unpredictable ways. This thesis takes advantage of the vast knowledge of the invasive Argentine ant, *Linepithema humile*, to focus on subtle and indirect effects on the recipient ecosystems, specifically on native vertebrates (amphibians, birds) in Doñana National Park (southern Spain). This tiny intruder is notorious for displacing the native ant community in ecosystems it has successfully invaded. Therefore, this thesis assesses whether the effects of potential prey depletion scale to higher trophic levels. Although the Argentine ant lacks recognized weapons, it is well known for its aggressiveness when outcompeting native ants. I evaluated whether native vertebrates at their most vulnerable stages are susceptible to being attacked or disturbed by the invasive ant and if they suffer from lethal or sublethal effects that hamper their development. These issues were addressed through field sampling and monitoring, field and laboratory experiments, and analyses of chemical, isotopic, histological, physiological, and behavioral parameters. My results reveal the Argentine ant invasion has negative impacts on native vertebrates in Doñana at different levels. For example, the native amphibians studied here have altered their diet, by shifting to non-ant preys in infested areas. Additionally, vertebrates in their early stages of development showed poor body condition under experimental (juvenile amphibians) and field (chicks) conditions when they were fed a diet supplemented with Argentine ants or raised in invaded areas. Furthermore, both myrmecophagous species, such as the natterjack toad, *Epidalea calamita*, and non-ant predators, such as the great tit, *Parus major*, modified their habitat use in invaded compared to uninvaded areas, although for different reasons (foraging and breeding, respectively). Finally, I demonstrate, for the first time in the literature, that the invasive Argentine ant has a powerful venom—iridomyrmecin—that is able to paralyze and kill native vertebrates, specifically juvenile amphibians. This unexpected finding deserves special attention, as it may play a key role in the previously demonstrated negative effects/invasiveness of this species. Overall, this thesis reveals overlooked indirect and subtle effects of an invasive species at different ecosystem levels, but also yields novel information about the mechanisms underlying these effects.

Resumen

Una de las principales causas de la pérdida de biodiversidad son las invasiones biológicas. A pesar de los avances en esta disciplina, aún se ignoran muchos de los impactos de estas especies, los cuales podrían vulnerar la estructura y función de los ecosistemas de un modo impredecible. Esta tesis aprovecha el amplio conocimiento que existe sobre la hormiga argentina, *Linepithema humile*, una de las peores especies invasoras conocidas, para centrarse en los efectos sutiles e indirectos que ocasiona en los ecosistemas nativos que invade, tomando como modelos anfibios y aves en el Parque Nacional de Doñana (sur de España). Esta diminuta intrusa es bien conocida por desplazar la práctica totalidad de las hormigas nativas en los ecosistemas donde invade de manera exitosa. Por ello, mi tesis analiza si la disminución potencial de presas afecta a niveles tróficos superiores. Aunque la hormiga argentina no cuenta con armas de defensa reconocidas, se sabe de su gran agresividad cuando se trata de eliminar a las especies de hormigas nativas. Mi tesis estudia si los vertebrados nativos, en sus estadios más vulnerables, son susceptibles de ser atacados o molestados por la hormiga invasora en Doñana y sufrir consecuencias letales o subletales en su desarrollo. Se abordan estas cuestiones mediante muestreos y seguimientos en campo, experimentos tanto en campo como en laboratorio, así como análisis de parámetros químicos, isotópicos, histológicos, fisiológicos y comportamentales. Los resultados ponen de manifiesto que la invasión de la hormiga argentina afecta negativamente a vertebrados nativos, y lo hace a diferentes niveles. Por un lado, la comunidad de anfibios nativos estudiados altera su dieta, reduciendo la cantidad relativa de hormigas que ingieren en las zonas infestadas de hormiga argentina y sustituyendo las hormigas por otros artrópodos. Por otro lado, vertebrados en sus estadios más vulnerables ven disminuido su desarrollo tanto en condiciones de laboratorio (juveniles de anfibios) como en el campo (pollos), al criarse en una dieta suplementada con hormiga argentina o en zonas invadidas. Además, tanto depredadores especialistas en hormigas —sapo corredor–, como especies no mirmecófagas –carbonero común–, ven alterado el uso del hábitat en zonas invadidas, tanto con el fin de forrajear o para reproducirse. Por último, he demostrado, por primera vez en la literatura, que la hormiga argentina cuenta con un veneno—iridomyrmecina— capaz de paralizar y matar vertebrados, concretamente juveniles de anfibios. Este inesperado hallazgo merece especial atención, ya que podría jugar un papel clave en los efectos negativos/poder de invasión ya demostrados en esta especie. Esta tesis revela impactos sutiles e inadvertidos de una especie invasora, a diferentes niveles en el ecosistema, además de profundizar en los mecanismos que los ocasionan.

Introduction

General introduction

Growing awareness of biodiversity loss

Human's footprint in the Globe has led to propose the terms "Anthrocene" (by Andrew Revkin in 1992) and "Anthropocene" (by Eugene Stoermer in the 1980s and popularized by Paul Crutzen in 2000) to coin the new geologic Era we are living after Holocene (Rull 2018; Wilson 2016). No other species but humans had ever modified the Earth in such a manner to cause the extremely high biodiversity loss in so little time (geological time). It is difficult to quantify extinction rate of the last century but it has been estimated between 10.000 and 50.000 species per year (27.000 by Edward O. Wilson, Delibes 2005), which equals a minimum of 27 species lost per day and one per hour. This rate is more than comparable to previous mass extinctions (Ceballos *et al.* 2015). We are not only on the side that suffer the consequences of such extinctions but on the side that originates them, emulating M. Delibes, we are *the meteorite* driving the planet to a sixth mass extinction (Delibes 2005).

From the mid-20th century, this concern was global in scope and materialized in international organizations devoted to preserve biodiversity by promoting legal instruments and specific research targets (Convention on Biological Diversity *CBD*; International Union for Conservation of Nature *IUCN*; Strategic Plan 2020-Aichi targets). From the 1970s, studies on global change drivers have also intensified, offering a broad perspective of human impacts on nature and revealing there is much to be done (Schlesinger 2006). In spite of all scientific efforts, biodiversity is declining at a faster rate than we are able to fully understand the mechanisms underlying their causes and consequences. This reason leads Edward O. Wilson, who promoted the term *biodiversity*, to suggest the protection of half-Earth as the real solution to prevent most of the species from extinction (Wilson 2016).

In fact, habitat destruction (human land use/land cover change) is one of the most important causes of biodiversity loss, together with climate change, overexploitation, and biological invasions, including pathogens (Sala *et al.* 2000; Tylianakis *et al.* 2008; Vitousek 1994; Wilson 2016). Drivers of biodiversity loss are not only diverse but also interconnected, increasing the variability of ecosystem responses and complicating the predictions of each driver's effect (Bradley *et al.* 2010; Brook *et al.* 2008; Didham *et al.* 2007; González-Varo *et al.* 2013; Sala *et al.* 2000).

Biological invasions as drivers of global change

Box 1. Personal considerations

In this thesis I use indistinctly the terms alien, exotic, introduced, nonindigenous and non-native species.

Because there is an ongoing debate on the definition of *invasive species* (Ricciardi *et al.* 2013), I specify here I refer to *any nonnative organism that enters a novel environment with human assistance, establishes a self-sustaining population, and spreads rapidly* as an invasive species.

Regarding the impact of invasive species, I would focus on the *ecological impact* and consider it as *any measurable and significant change on the properties or processes of an ecosystem by a non-native species, regardless of perceived value to humans* (unifying previous definitions given by Ricciardi *et al.* 2013 and Simberloff *et al.* 2013). I also use the terms *impact* and *effect* with the same purpose.

Biological invasions originate in the worldwide travels, which allow transportation of species to remote places that would never be reached without human help. Species translocation started to be significant at the end of the 18th century with the European world colonization and became a serious threat with the globalization and the international trade at the end of the 20th century. Human traveling is likely to involve more than one species, both intentionally and accidentally. Attractiveness for exotic and rare species, together with desire of possession have likely encouraged transportation (Courchamp *et al.* 2006; Hulme *et al.* 2008; Pierce *et al.* 2003). For example, exotic species serve as ornaments (e.g. Rosaceae, Pyšek 1998), pets (e.g. Psittacidae, Lockwood 1999), and prey on sport hunting or fishing (e.g. red swamp crayfish, Clavero 2016). Besides, exotic species can also become valuable resources previously unavailable (Pejchar and Mooney 2009; Shackleton *et al.* 2018). Although introduction of exotic species may have benefits for some stakeholders, it has been amply demonstrated to have an impact in both short and long term in the novel ecosystems with devastating consequences (Clavero and García-Berthou 2005; Crooks 2002; Jeschke *et al.* 2014; Mack *et al.* 2000; Vilà *et al.* 2011). Biological invasions alter native ecosystems by direct predation, niche displacement, competitive exclusion, hybridization and introgression, and parasitism, on native species (Kenis *et al.* 2009; Mack *et al.* 2000; Mooney and Cleland 2001). But the effects on native species can also be indirect by modifying environmental characteristics, inducing behavioral changes or producing long-term cascading effects (Blackburn *et al.* 2014; David *et al.* 2017; Kenis *et al.* 2009; Mack *et al.* 2000). As major international concern, prevention, control and eradication of the most harmful invasive species constitutes the 9th Aichi Biodiversity Target within the Strategic Plan for 2020 of the CBD (Strategic Plan 2020-Aichi targets, target 9).

Invasion Ecology: from the origins to the gaps

The study of biological invasions is not a new phenomenon. Charles Elton in his famous volume *The Ecology of invasions by animals and plants* (1958), the most cited source in the field (Richardson and Pyšek 2008), already predicted the discipline and set out concern on the consequences of biological invasions (Simberloff 2011). Invasion ecology has grown in the last decades into a consolidated multi-disciplinary ecology field. On one hand, species introduction are unplanned experiments that allow addressing ecological questions on species' natural history, interactions and evolution (Meyerson and Mooney 2007). On the other hand, biological invasions are one of the main drivers of global change and biotic homogenization, and their causes and consequences on ecosystems should be studied (Bellard *et al.* 2016; Hobbs and Mooney 2000; Holway and Suarez 2006; Mack *et al.* 2000; McKinney and Lockwood 1999; Ricciardi 2007; Vitousek *et al.* 1996).

Albeit of the progress, the mechanisms and consequences of biological invasions remain largely unknown (Kumschick *et al.* 2015; Pyšek *et al.* 2008; Ricciardi *et al.* 2013; Richardson 2011; Simberloff *et al.* 2013). This is partially due to the difficulty in assessing the impact on native ecosystems, especially when subtle, unsuspected and/or delayed (Blackburn *et al.* 2014; Crooks 2005; Simberloff *et al.* 2013). Such overlooked effects are common and potentially impinge structure and function of ecosystems in unpredictable ways (Crooks 2002; Gozlan *et al.* 2005; Mack and D'Antonio 1998; Rowles and O'Dowd 2009; Simberloff 2011). To fill these gaps, a broader scope is needed. For example, switching to a more holistic approach by considering ecological impacts as a continuous response rather than dichotomous, or by considering not only invading species traits but also those of the recipient community and their interactions (Catford *et al.* 2009; Pyšek *et al.* 2012; Ricciardi *et al.* 2013). Once impacts at community level on one taxa are recognized, it becomes essential to explore potential effects on other different taxa at the ecosystem level. Far from one-sided, effects of invasive species should be also looked at multiple sides (Pintor and Byers 2015;

Ricciardi *et al.* 2013). Introduced species necessarily generate new ecological relationships. Impact on native species has been mainly addressed by considering invasive species as a predator (top-down) (Blackburn *et al.* 2004; Courchamp *et al.* 2000; David *et al.* 2017; Mollot *et al.* 2017; Salo *et al.* 2007). However, the opposite perspective, by considering invasive species as prey, has received much less attention (bottom-up effects) (David *et al.* 2017; Pintor and Byers 2015). Finally, there are other biases in the study of invasion ecology, such as taxonomic and geographical (McKinney and Lockwood 1999; Pyšek *et al.* 2008). Expanding the already gathered knowledge to the understudied taxa and areas would likely improve generalizations and provide new insights on the invasion ecology. Nevertheless, taking advantage of the vast knowledge of the most studied invasive species may help to unravel unexpected and subtle effects.

The role of ants in biological invasions

Ants constitute a suitable model to approach invasion ecology due to the variety of interactions they establish and the functions they play in the ecosystems, together with their broad geographical distribution in most terrestrial habitats. Ants are one of the most diversified animal taxa, with more than 16,100 described species so far (AntWeb). Ants are soil engineers and favor soil turnover but also act as predators, herbivores, granivores, scavengers and detritivores (Hölldobler and Wilson 1990). They also establish a wide array of relationships, including mutualisms with plants and other insects, contributing to seed dispersal and pollination (Ness *et al.* 2010). Besides, ants serve as prey of specialist predators including amphibians (Berazategui *et al.* 2007), reptiles (Pianka and Parker 1975), mammals (Naples 1999), birds (Wiebe and Gow 2013), and other invertebrates (Gotelli 1996), and are also host of many parasitoid species (Feener and Brown 1997; Lachaud and Pérez-Lachaud 2012). Because of their pervasiveness and ecological functionality, it is not surprising that they have been considered good indicators of ecosystem change and recently suggested to help in the restoration of degraded areas (Andersen and Majer 2004; Majer and Nichols 1998; Moranz *et al.* 2013). However, many aspects remain to be studied, from taxonomic to physiological, behavioral and ecological diversity (Frank *et al.* 2017; LeBrun *et al.* 2014; Shorter and Rueppell 2012)

Ants' worst enemies are other ants

In addition to abiotic factors, several biotic determinants influence ant species distribution and abundance such as the effect of other ant species within the same community, so that each species abundance may be regulated by the ant species' assemblage itself (Cerda *et al.* 2013). Invasive ants often disrupt native ant communities and reduce their diversity with obvious implications on other organisms. There are at least 147 introduced ant species (with established populations) (McGlynn 1999), 19 of which are included in the Global Invasive Species Database (GISD). Besides, Bertelsmeier *et al.* (2017) identified nine other ant species as potential invaders based on their traits. Furthermore, from 17 invertebrate species in the list of "100 of the world's worst invaders", five are ants (GISD; Lowe *et al.* 2000). Three of them belong to the ant subfamiliy Myrmicinae, the red imported fire ant (*Solenopsis invicta*), the big-headed ant (*Pheidole megacephala*) and the little fire ant (*Wasmannia auropunctata*); the yellow crazy ant (*Anoplolepis gracilipes*) is a Formicine and the Argentine ant (*Linepithema humile*) is a Dolichoderine.

In addition to the common traits shared with other invasive species such as being generalist and easily dispersed by humans (McKinney and Lockwood 1999), invasive ants are generally polygynous, small in size, highly aggressive, propagated by budding and able to thrive in disturbed areas (peripatetic) (Hölldobler and Wilson 1990; Holway *et al.* 2002; Passera 1994). Besides, the key success of (most) invasive ants is their cooperative behavior among workers from different nests (Hölldobler and Wilson 1990; Holway *et al.* 2002; Passera 1994). This social structure is known as unicoloniality and results into numeric dominance and superior competitive abilities over native ant species, which has been mainly studied with the Argentine ant as model species (Carpintero and Reyes-López 2008; Holway 1999; Morrison 2000; Rowles and O'Dowd 2007; Walters and Mackay 2005).

Due to the mentioned ecological functionality of ants, invasive ants do not only alter the native ant community but also negatively affect other organisms both directly and indirectly, from individual to ecosystem levels, eventually inducing important cascading effects with catastrophic consequences (see reviews in Holway *et al.* 2002; Lach and Hooper-Bui 2010). Besides, once established, although there have been recently some successful cases, eradication of invasive ants in natural areas without impact on non-target species is almost impossible due to the use of toxicants, many of which were made for agricultural or urban scenarios (Hoffmann *et al.* 2016; Hoffmann *et al.* 2010; Oi and Drees 2009; Spring *et al.* 2017, but see Diaz *et al.* 2013 for manual removal of nests in a certain season).

The Argentine ant as study model

The Argentine ant, *Linepithema humile* (Mayr 1868), formerly *Iridomyrmex humilis*, is one of the worst invasive species (Lowe *et al.* 2000; Luque *et al.* 2013) and also one of the most studied (the second after the zebra mussel, Pyšek *et al.* 2008). It has been used to examine a variety of ecological issues across different continents (Pyšek *et al.* 2008). It is native from the Paraná and Uruguay river basins in South America, which include the countries of Brazil, Argentina, Uruguay and Paraguay, and it has been introduced unintentionally in Mediterranean ecosystems all over the world where it has striking success (Espadaler and Gómez 2003; Giraud *et al.* 2002; Suarez *et al.* 2001; Van Wilgenburg *et al.* 2010; Vogel *et al.* 2010; Wetterer *et al.* 2009; Wild 2004).

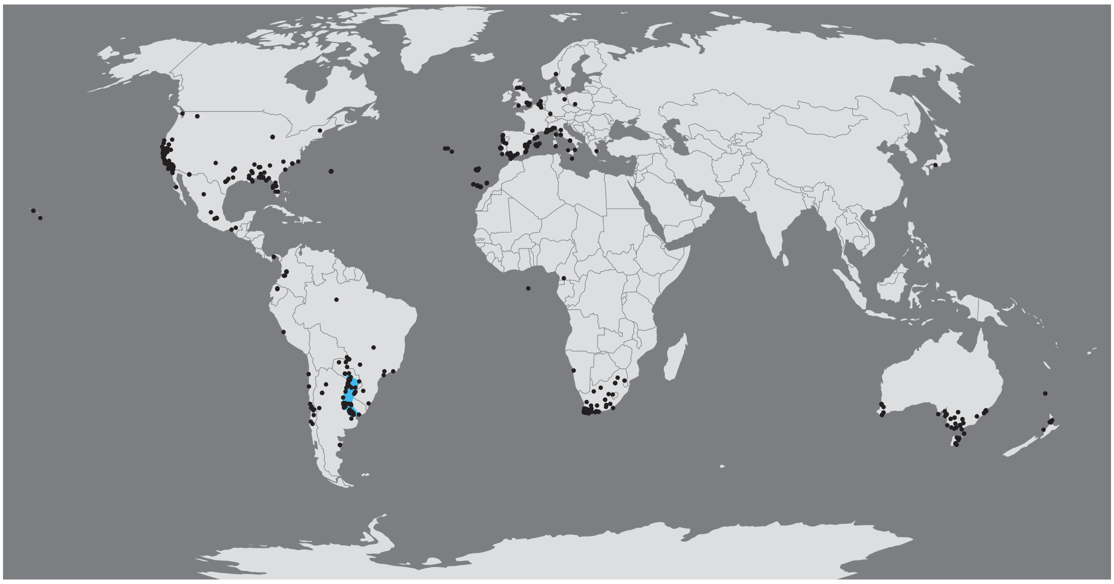


Fig. 1. Worldwide distribution of the Argentine ant (native range in blue) (see 2.10 in Material and Methods for further details).

In Europe, the Argentine ant was detected for first time in the mid-19th century in Madeira island, center of commerce between South America and Portugal, and at the end of the century it was found in continental Europe (Portugal, Martins 1907; Schmitz 1897; Wetterer and Wetterer 2006). Over the course of the 20th century the Argentine ant has spread mainly along the Mediterranean (but also Atlantic) coast. In Spain, it was first detected in Valencia in the 1920s (Font-de-Mora 1923; García-Mercet 1923).

Fig. 2. Argentine ants are barely 4 mm and lack a functional stinger. Due to their numerical superiority and aggressiveness they are able to subdue native ants of much larger size, such as this harvester ant of the genus *Pogonomyrmex* (*Photo credits: @alexanderwild.com*).

Where it is successful, the Argentine ant holds high-density colonies of polygynous nests that lack intraspecific competition (unicoloniality, Helanterä *et al.* 2009; Suarez *et al.* 2008). Their abundance, together with their aggressiveness and foraging efficiency contribute to disrupt the native ant community by means of interference and exploitative competition (Abril and Gómez López 2009; Angulo *et al.* 2011; Carpintero *et al.* 2007; Gordon and Heller 2014; Holway 1999; Sanders *et al.* 2003; Suarez *et al.* 1998; Tillberg *et al.* 2007, Fig. 2). Negative effects of the Argentine ant on ant communities have been studied in great detail in California and Europe (e.g., Carpintero *et al.* 2005; Gordon and Heller 2014). Once the Argentine ant has established successfully, it displaces almost all native ant species (Angulo *et al.* 2011; Carpintero *et al.* 2005; Holway and Suarez 2006; Suarez *et al.* 1998). Despite their small size, they also attack in mass nests of larger native ants that do not neccesarily share the same food resources, such as *Cataglyphis floricola*, *Messor lusitanicus*, *Pogonomyrmex subnitidus*, *P. californicus*, *P. rugosus*, and *Veromessor andrei* (Angulo *et al.* 2007; Zee and Holway 2006, S. Castro-Cobo and J. M. Vidal-Cordero pers. comm.). Although the Argentine ant is omnivorous, it has a strong preference for sweet substances that gets from farming mealybugs in shrubs and trees, especially in the introduced range, the reason why is known as *sugar ant* (Holway *et al.* 2002; Newell and Barber 1913; Tillberg *et al.* 2007). Therefore, it replaces native ants not only on the ground but also on the trees (Angulo *et al.* 2011; Carpintero *et al.* 2005; Wetterer *et al.* 2001).

The consequences of modifying the native ant communities have been largely demonstrated, from agricultural ecosystems (i.e. orange orchards) where they constitute a pest due to the mutualism with honeydew-secreting insects, to natural ecosystems where they displace other invertebrates impinging on ecosystem processes such as scavenging, plant pollination, and seed dispersion (see reviews in Holway *et al.* 2002; Lach and Hooper-Bui 2010, and also Angulo *et al.* 2011). However, contrary to other invasive ants (e.g., *Solenopsis invicta*), negative impacts have focused mainly on the native ant community or other invertebrates, but studies about negative impacts on vertebrates are relatively scarce (Table 1).

Table 1. Reported effects of invasive Argentine ants (*Linepithema humile*) on native vertebrate species. We provide: location of the study (Location), study date (Date), whether comparisons between invaded and uninvaded areas or treatments were done (Vs, yes/no), whether negative effects were reported (Effect, yes/no), whether predation to the Argentine ant was attributed (Pred, yes/no). We briefly describe the reported effect and observations when necessary. References are sorted by publication year.

Species	Location	Date	Vs	Effect	Pred	Reported effect	Observations	Ref
AMPHIBIANS & REPTILES								
Japanese treefrog, *Hyla japonica*	Hiroshima Japan	2001	no	no	no	None	Argentine ants are the most abundant ant in the stomachs of juveniles in invaded areas	Ito *et al.* 2009
Gecko	Doñana, Spain		no	yes	yes	Kill and prey some small gecko	Field observation	Delibes 2005
Coastal horned lizard, *Phrynosoma coronatum blainvillei*	California, USA	1995-1998	yes	no	no	Lower abundance in invaded areas	Field sampling	Fisher *et al.* 2002
	California, USA	1999	yes	no	no	Lower growth rate of hatchlings on a *L. humile* diet	Diet lab experiment	Suarez and Case 2002
	California, USA	1996-1997	yes	yes	no	Predator shifts to non-ant prey in invaded areas	Scat-analyses. Low preference for Argentine ant in the lab	Suarez *et al.* 2000
Several reptiles and amphibian	California, USA	1997	no	yes	yes	Observations of reptiles swarmed and eaten by Argentine ants	Tropical captive animals in collections; some individuals died days after contact with ants	Lemm 1997

Table 1. (Continued)

Species	Location	Date	Vs	Effect	Pred	Reported effect	Observations	Ref
BIRDS								
	Rapa Nui, Pacific Island	2016	no	no	no	Argentine ants recruited to pipped eggs	Field experiment of Argentine ant recruitment to domestic hen eggs	Varela *et al.* 2018
Various humming-birds species	California, USA	2014	yes	yes	no	Birds avoid feeding on nectar with Argentine ants	Field observations and aviary experiment. Taste and odour cues. Interference competition.	Rankin *et al.* 2018
Bulwer's petrels, *Bulweria bulwerii*	Madeira, Portugal	2006-2017	no	yes	yes	Sporadic predation of Argentine ants on hatchlings but no effect on breeding success	Recent decrease in Bulwer's petrel breeding success coincident with the introduction of the Argentine ant.	Boieiro *et al.* 2018
Red-tailed tropicbirds, *Phaethon rubricauda*	Rapa Nui, Pacific Island	2014-2015	no	yes	yes	Disturbed adults and a chick partially predated by ants	Direct observations. Argentine ants were present in all nests	Flores *et al.* 2017
Tropicbird, *P. lepturus catesbyi*	Bermuda, UK	2013-2015	no	yes	yes	Five cases of nestlings mortality (one in 2014, four in 2015)	Attributed predation to Argentine ants when ants were found over dead nestlings	Mejías *et al.* 2017
Blue tit, *Cyanistes caeruleus*	Cataluña, Spain	2005-2007	yes	no	no	No effect on breeding and offspring quality	Resource competition (for caterpillar, Estany-Tigerström *et al.* 2010)	Estany-Tigerström *et al.* 2013
Insectivorous birds	Cataluña, Spain	2005-2007	yes	no	no	No direct effect on bird abundance	Resource competition	Pons *et al.* 2010
Barn swallow, *Hirundo rustica*	Doñana, Spain		no	yes	yes	Kill and prey two chicks on their nest	Field observation	Delibes 2005
Hawaiian goose, *Branta sandvicensis*	Hawaii, USA		no	yes	yes	Recruit heavily to pipped eggs and attack emerging chicks	This paper is a review and referred to this data by F. Duvall pers. comm.	Krushelnycky *et al.* 2005
Dark-eyed junko, *Junco hyemalis*	California, USA	1998-2000	no *	yes	yes	Kill and responsible for < 2% of failed nests. Argentine ants recruit to experimental pipped eggs	Attributed predation if ants were over nestlings that were still living or were eating nestlings that were alive the previous visit	Suarez *et al.* 2005
California least tern, *Sterna antillarum browni*	California, USA	1989-1994	no	yes	yes	Attack and kill chicks	Field observation along a study about southern fire ant effects, *Solenopsis xyloni*	Hooper-Bùi *et al.* 2004
Least bell's vireo, *Vireo bellii pusillus*	California, USA	2000	no	yes	yes	Prey upon hatchlings in a nest and caused another to fledge 1–2 days early	Video. Ants recruited during hatching. Nestlings were bitten repeatedly.	Peterson *et al.* 2004
Hawaiian dark-rumped petrel, *Pterodroma phaeopygia sandwichensis*	Hawaii, USA	1994	yes	no	no	No effect in nesting success between invaded and uninvaded areas	Cold temperatures within the petrel burrows discourage heavy ant foraging to the nest chambers	Krushelnycky *et al.* 2001
California gnatcatcher, *Polioptila melanura*	California, USA	1994-1995	no	yes	yes	1.2% chicks predation	Two nests with many Argentine ants were considered to have been depredated	Sockman 1997
MAMALS								
Shrews, *Notiosorex crawfordi*, *Sorex ornatus*	California, USA	1995-1999	yes	yes	no	Negative effect on *N. crewfordi* and no effect on *S. ornatus* abundance.	Field sampling. Cold soil temperatures inhibit ant foraging into the deeper parts of the burrows	Laakkonen *et al.* 2001

* Comparison was made to assess recruitment of ants to experimental eggs but there was no comparison in the chicks monitoring.

Goals

This thesis explores the effect of the invasive Argentine ant on native vertebrates. The main goal is to increase the understanding of the mechanisms underlying the biodiversity loss, trying to fill some of the current gaps in invasion ecology above-mentioned. On one side, I focus on bottom-up effects of invasive species, which have received low attention (Section 1, Section 3). On the other side, I focus on unexpected, subtle, overlooked impacts to understand their causes and consequences (Section 2, Section 4). I use different empirical and experimental approaches to obtain data from the field and laboratory conditions, measuring diverse chemical, physiological, behavioral and ecological parameters. Finally, I address the effects of the Argentine ant at different levels of organization, including individual (Section 3), population (Section 4) and community (Section 1) levels. I focus on native vertebrates that live in the invaded ecosystems, using amphibians and birds as study models. My work is developed from two perspectives: First, from the vertebrates' point of view, as being native predators that will likely suffer from the prey depletion associated to the Argentine ant invasion or directly exploit it as prey; Second, considering native vertebrates during their most vulnerable stages (reproduction or early developmental stages), as being susceptible to lethal or sub-lethal effects from the invasive ant.

This general objective has been addressed through the following specific goals:

1) Impact of an invasive prey on native predators (Section 1):
    i. To propose a theoretical framework that explains the effect of an invasive prey on native predators, by expanding current hypotheses that focus on the success or failure of an introduced prey in relation to native predators.
    ii. To evaluate the bottom-up effect of the invasive prey on an amphibian community, considering the degree of ant specialization of the native predators.
2) The study of the Argentine ant venom (Section 2):
    i. To assess the differential effect of the invasive ant on three species of newly emerged amphibians.

   ii. To identify and characterize the mechanism of attack the Argentine ant use towards vertebrates comparing to its “sibling” native ant.
   iii. To quantify the potential global impacts on amphibian species, which distribution ranges potentially overlap with those of the Argentine ant.

3) Effects on growth and survival:
   i. To evaluate the effects of feeding on Argentine ants on the growth and survival of metamorphosed amphibians (Section 3).
   ii. To assess the effects of breeding by native avian species on Argentine ant invaded sites (Section 4).

In the subsequent sections, I describe the framework, justification, and specific hypotheses and predictions of these goals.

Section 1: Native predators living in invaded areas: responses of terrestrial amphibian species to an Argentine ant invasion

The vulnerability of native communities to invasions of nonnative species depends on their ecological resistance, which is mainly defined by the presence of native competitors and predators (Ricciardi *et al.* 2013). Predators can promote resistance through a variety of mechanisms, including their abundance, their recognition and consumption of invasive prey, their functional response to invasive prey, and their ability to respond over time (Carlsson *et al.* 2009; Carthey and Banks 2014; Catford *et al.* 2009; Twardochleb *et al.* 2012).

For example, the well-known enemy release hypothesis (as well as its variants, such as the enemy reduction hypothesis and the enemy inversion hypothesis; Catford *et al.* 2009) states that exotic species can become invasive because they lack coevolved enemies in their introduced ranges (Colautti *et al.* 2004; Keane and Crawley 2002; Sih *et al.* 2010). In such situations, native predators do not limit the invasion, because predation does not occur or only occurs at low levels. In contrast, the more recently published exotic prey naïveté hypothesis and the increased susceptibility hypothesis posit that the naïveté of introduced prey species means they experience higher predation pressures than do native prey species (Catford *et al.* 2009; Colautti *et al.* 2004; Li *et al.* 2011; Wanger *et al.* 2011). In this case, native predators should be able to control the invasion by predating upon the introduced prey species, acting as a form of biotic resistance. The specialist–generalist hypothesis states that invasion success should be minimized when predators are generalists because they would, thus, be able to consume introduced prey (Catford *et al.* 2009; Sax *et al.* 2007). Although dietary specialization actually exists along a gradient, Catford *et al.* (2009) distinguish two extremes: the absolute generalist, which interacts with any and all species, and the absolute specialist, which preys upon a single species. However, if the invasive prey species can defend itself chemically (e.g., with toxins), then it could escape even predation by generalists. Such a situation is described by the novel weapons hypothesis—the competitive ability of invasive prey would, therefore, be enhanced (Callaway and Ridenour 2004; Carlsson *et al.* 2009; Ricciardi *et al.* 2013).

These hypotheses are rather one-sided in that they largely focus on the success of the introduced prey species without addressing effects on native predators (but see Pintor and Byers 2015). By considering the effects on predators in each of these scenarios, we can establish a theoretical framework for understanding how invasive prey affects native predators. Here, we have expanded the hypotheses described above to address the effects of introduced prey on predators with different degrees of dietary specialization. According to the exotic prey naïveté and the increased susceptibility hypotheses, predators may benefit from the large availability of naïve prey because they can consume them (Cabrera-Guzmán *et al.* 2012; Glenn and Holway 2008; Monzó *et al.* 2013; Wanger *et al.* 2011). Based on the specialist–generalist hypothesis, generalist may benefit more than specialist predators (Catford *et al.* 2009; Maerz *et al.* 2005; Sax *et al.* 2007). And based on the enemy release hypothesis native predators would benefit more if the introduced prey species were relatively similar to native prey species (Carlsson *et al.* 2009; Robbins *et al.* 2013). Finally, according to the novel weapons hypothesis, predators will not benefit if the invasive prey releases toxins. Although native predators could develop ways for dealing with toxins, this process is expected to occur over the long term (Carlsson *et al.* 2009). Different degrees of naïveté have been observed in prey (Banks and Dickman 2007) and may also exist in native predators. In this sense, Bytheway *et al.* (2016) have shown how behavioral flexibility on the part of invasive predators can enable invaders to respond rapidly to novel situations. However, such behavioral flexibility should be less common in native predators (Carlsson *et al.* 2009): the predator may not recognize a new prey species; it may recognize an invasive species as prey but fail to capture it; or it may capture it without consuming it. Taken together, these hypotheses suggest that native predators are more likely to be negatively impacted under the following conditions: they have a more specialized diet; the invasive prey species differs from the native prey species included in their diets; or the invasive prey species releases toxins.

Ants play crucial ecological roles within ecosystems (Lach *et al.* 2010) and, consequently, the negative effects of invasive ants can scale up to higher trophic levels (Holway *et al.* 2002). The effects of the Argentine ant on non-ant species, including ant specialist predators, have been studied in California and Japan (e.g., Suarez and Case 2002; Touyama *et al.* 2008). Unlike other invasive ants (e.g., *Solenopsis invicta*, *Wasmania auropunctata*), which have a venomous stinger, the Argentine ant does not possess a functional stinger that it could use to defend itself from predators or to subdue vertebrate prey (Holway *et al.* 2002). Although the Argentine ant can prey on nestlings of some bird species, it has not been considered to be a serious threat (Estany-Tigerström *et al.* 2013; Estany-Tigerström *et al.* 2010; Hooper-Bui *et al.* 2004; Sockman 1997; Suarez *et al.* 2005). When it comes to native predators in general, negative effects related to prey displacement have been observed for the ant-eating specialist *Phrynosoma coronatum*, the coastal horned lizard (Suarez and Case 2002; Suarez *et al.* 2000); conversely, some ant-eating invertebrates appear to benefit from the presence of this invasive ant (Glenn and Holway 2008; Touyama *et al.* 2008). These contrasting results suggest that dietary specialization is not the only factor driving the impacts of the Argentine ant on predators.

In this study, we examined the threat posed by the Argentine ant to a native amphibian community. Amphibians are the world's most vulnerable group of vertebrates (accounting for ~41% of endangered species; Hoffmann *et al.* 2010). Terrestrial amphibians are known to consume large quantities of ants (Table 2) and are, thus, potentially vulnerable to Argentine ant invasions. We aimed to answer three key questions. First, is amphibian abundance different in invaded areas because it is dependent on dietary specialization? We predicted that the greater a species' dietary specialization on ants, the more its abundance would decrease in invaded areas. Second, is ant availability similar in invaded and uninvaded areas, and do predators with different dietary specializations track ant availability differently? We predicted that ant consumption would track ant availability for generalist predators but would decrease for the most specialized predators. Because no other ant species are available in invaded areas, predators could compensate by consuming prey of other taxa (i.e., by excluding ants from their diets). Then, if a dietary shift was to occur, the predators' nitrogen isotopic values would be expected to differ because the nitrogen isotopic value of a predator reflects that of its prey (Post 2002). Third, do amphibian ant specialists prefer native ants to Argentine ants? Is this preference affected by ant morphology or by prior exposure to the Argentine ant? We predicted that the consumption of Argentine ants by amphibian ant specialists would depend on the degree of similarity between the Argentine ant and the native ant species consumed by the specialist. We also predicted that prior exposure to the invader could alter consumption patterns in one of two ways: (a) consumption could increase relative to a naïve individual if the encounter resulted in the amphibian learning to recognize the Argentine ant as prey or (b) consumption could decrease relative to a naïve individual if the prior exposure resulted in a negative experience and the amphibian learned to avoid eating Argentine ants.

Fig. 3. The natterjack toad, *Epidalea calamita*, is an active-search hunter highly myrmecophagous. (*Illustration: Dailos Hernández-Brito*).

Table 2. Percentage of Formicidae in the diet of four Mediterranean amphibian species in Spain. The percentage provided is the mean (± SE, when available); N is the number of individuals analyzed. Locations are specified, as are the relevant references. When the data came from this study, they are coded as INV for invaded areas and UNI for uninvaded areas.

Species	Formicidae (%)	N	Location	Reference
Epidalea calamita	64.22	8	Almería	Valverde 1967
	0	2	Doñana	Valverde 1967
	72	62	Southern Spain	López Jurado 1982
	25.49	2	Salamanca	Lizana *et al.* 1986
	High abundance	-	Salamanca	Lizana & Pérez-Mellado 1990
	19.50	10	Cataluña	Bea *et al.* 1994
	43.16 ± 4.44	27	Doñana - INV	This study-Section1
	47.91 ± 5.09	35	Doñana - UNI	This study-Section 1
Hyla meridionalis	58	105	Canarias	Cott 1934
	Greatest abundance	5	Almería	Valverde 1967
	21.75	77	Granada	Hodar 1991
	32.64 ± 24.78	3	Doñana - INV	This study-Section 1
	33.30 ± 11.10	6	Doñana - UNI	This study-Section 1
Discoglossus galganoi	16.71*	43	Salamanca	Lizana *et al.* 1986
	10.08*	80	Visma, Galicia	Vázquez 1999
	12.6*	12	Isla de Sálvora	Galán 2003
	15.97 ± 8.63	6	Doñana - INV	This study-Section 1
Pelobates cultripes	2	12	Doñana	Valverde 1967
	4.35	18	Doñana	Díaz-Paniagua 2005
	0.42 ± 0.42	16	Doñana - INV	This study-Section 1
	24.32 ± 15.93	8	Doñana - UNI	This study-Section 1

*While all hymenopterans were counted, it was noted that most belonged to Formicidae.

Section 2: Are amphibians threatened by a global invasive ant?

Biological invasions have major impacts on ecosystem functioning and are among the main cause of biodiversity loss worldwide. Recently, invasive species have been categorized based on their impacts (Blackburn *et al.* 2014), and strategies for recognizing high-impact species have been proposed (Kumschick *et al.* 2015). However, three main issues hamper our ability to accurately predict a given species' impact. First, some of the pervasive effects of invaders are unexpected and not readily detected (Simberloff *et al.* 2013). Second, research on the impact of invaders is highly skewed toward certain taxa or certain biological traits (Bellard and Jeschke 2016; Kumschick *et al.* 2015; Pyšek *et al.* 2008; Seebens *et al.* 2018). Third, a lack of mechanistic information on how an invader interacts with native taxa may lead to a species being placed in a lower impact category than might otherwise be the case (Blackburn *et al.* 2014).

Five ant species are among the worst invaders (Lowe *et al.* 2000) and, broadly speaking, the mechanistic underpinning for their impacts is competition (exploitative and/or interference) with native species within ant communities, which results in whole-ecosystem changes. Three of these top invasive ants also have negative consequences due to opportunistic predation, poisoning, or toxicity; they are the red imported fire ant, *Solenopsis invicta*, the little fire ant, *Wasmannia auropunctata*, and the yellow crazy ant, *Anoplolepis gracilipes* (GISD; Holway *et al.* 2002). The impacts of the Argentine ant also ripple up to higher trophic levels—by affecting ant predators, mainly through dietary shifts driven by changes in the ant community (Alvarez-Blanco *et al.* 2017 = Section 1; Suarez and Case 2002). While the invasive ant *S. invicta* preys on vertebrates (Allen *et al.* 2004), only a few studies suggest that *Linepithema humile* may occasionally prey on nestlings of ground-nesting birds (Boieiro *et al.* 2018; Flores *et al.* 2017; Hooper-Bui *et al.* 2004; Sockman 1997; Suarez *et al.* 2005). Unlike *L. humile*, *S. invicta* has a stinger and injects venom in its prey (Langkilde 2009). *L. humile* lacks visible weapons (e.g., a functional stinger or large mandibles), but a recent study established that it nonetheless deploys defensive compounds against native ants (Welzel *et al.* 2018).

Here, we show how the use of toxic chemicals by *L. humile* can be lethal to juvenile terrestrial amphibians of three species, a group that includes the ant's main potential predators. To characterize the magnitude of *L. humile*'s impacts, we estimated levels of ant aggression directed at amphibians, the composition and quantities of the toxin used, and the mechanisms of toxicity in juvenile amphibians. To determine the potential scope of the threat faced by amphibians, we employed global databases to estimate the number of terrestrial amphibian species whose ranges and habitats overlapped with those of the Argentine ant. We then looked at the IUCN Red List status of those species. Extrapolating from the toxicity results and the amphibian distribution analysis, there is strong evidence suggesting that this ant could cause major ecosystem impacts at higher trophic levels via toxicity-mediated predation, a previously unsuspected mechanism with serious implications for amphibian conservation.

Fig. 4. Dolichoderine ants have a highly developed pygidial gland. Iridomyrmecin is released from the gland reservoirs of the Argentine ant in droplets (*Illustration: María León*).

Section 3: Survival and growth of native toadlets feeding on an invasive ant prey

Biological invasions disrupt trophic relationships, causing negative effects on native ecosystems. Because predator-prey interactions are major drivers of ecosystem dynamics (Sergio *et al.* 2006), they also represent an essential aspect of invasions. Invasive species frequently alter existing predator-prey interactions, which may have unexpected effects on the ecosystem (Carlsson *et al.* 2009; Caut *et al.* 2008). The most harmful effect of invasive species, causing local species extinction, is through direct predation (David *et al.* 2017). Thus, a great deal of theoretical work has focused on the impacts of invasive predators on native preys (Ricciardi *et al.* 2013). In contrast, less work has examined the effects of invasive prey species on native predators. Pintor and Bayes (2015) performed a meta-analysis to quantify the global effects of the non-native preys on the native predator abundance as well as on predator fitness parameters, such as their survival and growth rate. While they found that the abundance of predators generally increased following prey invasion, a less clear pattern appeared when examining fitness parameters.

Several scenarios are possible regarding the effect of invasive prey on fitness of native predators, resulting in either positive or negative outcomes for predators. Invasive prey can thus benefit native predators if these can learn or adapt to exploit it as a suitable resource (Carlsson *et al.* 2009; Pintor and Byers 2015). Some predators benefit from the higher abundance of invasive prey, like the native purple martin (*Progne subis*), which increases its foraging efficiency by feeding on the red imported fire ant (*Solenopsis invicta*) during their mating flights (Helms *et al.* 2016). Similarly, the native woodland salamander (*Plethodon cinereus*) experienced higher growth in the presence of the invasive earthworm (*Lumbricus terrestris*), considered a suitable prey item (Ransom 2012). Other predators showed higher developmental rates when feeding on invasive prey rather than on native ones. Such is the case of the pit-building antlion (*Myrmeleon*), which experiences higher growth rates and develops longer mandibles when feeding on the Argentine ant (*Linepithema humile*) compared to native ants (Glenn and Holway 2008). Net increases in native predator abundance can also occur in the presence of invasive prey, as in the case of the jumping spider *Siler cupreus* in the presence of invasive Argentine ants (Touyama *et al.* 2008).

Alternatively, the presence of invasive prey can be detrimental to native predators. For example, the population of the specialist coastal horned lizard (*Phrynosoma coronatum blainvillei*) was shown to decline when feeding on the invasive Argentine ant (Suarez and Case 2002; Suarez *et al.* 2000). Some predators have also been found to be less abundant in invaded areas, as is the case for natterjack toads (*Epidalea calamita*) inhabiting areas invaded by the Argentine ant (Alvarez-Blanco *et al.* 2017 = Section 1). Recently, Langkilde *et al.* (2017) reviewed the costs that native predators suffer as a consequence of native prey being replaced by invasive species. These include reduced reproductive input, increased energy expenditure and exposure to unknown toxins or venoms from the invasive prey. Pintor and Bayes (2015) suggested that one of the key determinants of negative impacts of invasive prey on native predators consists in the prey having a low nutritious value. But it could also be the case that invasive prey has toxins or venoms (Callaway and Ridenour 2004; Carlsson *et al.* 2009; Ricciardi *et al.* 2013), which constitute a chemical defense in their native range (Berenbaum 1995; Uemura *et al.* 2017). Toxic invasive prey can thus take advantage of their chemical defenses in the invasive range, benefiting from the lack of coevolution with native predators and the resulting predator naivety to the prey chemicals (Hagman *et al.* 2009; Sih *et al.* 2010). This is the case of the invasive cane toad (*Rhinella marina*), introduced in Australia as pest control, and exhibits lethal toxicity to many native predators including snakes (Jolly *et al.* 2016), frogs (Crossland 2000), and crocodiles (Letnic *et al.* 2008). A second example is the red imported fire ant, which acts as both predator and prey to native eastern fence lizards (*Sceloporus undulatus*). When eaten, these ants sting lizards in their mouths with potentially fatal results (Langkilde and Freidenfelds 2010). In both cases, native predators have evolved or learned morphological or behavioral mechanisms to avoid envenomation (Freidenfelds *et al.* 2012; Langkilde 2009; Long *et al.* 2015; Robbins and Langkilde 2012). Such defensive mechanisms may incur in indirect costs such as increased energy expenditure (Ligon *et al.* 2012; Long *et al.* 2015) or increased predation risk from other predators (Orrock and Danielson 2004).

The Argentine ant is the main ant available for predators in invaded areas (Alvarez-Blanco *et al.* 2017 = Section 1; Angulo *et al.* 2011; Carpintero *et al.* 2007; Holway *et al.* 2002). It has recently been shown that the Argentine ant possesses chemical compounds to repel other ant species (Welzel *et al.* 2018). Moreover, these chemical compounds are also used to kill newly emerged toadlets in invaded areas (Section 2). The venom is released over the toadlets, who absorb it through their permeable skin, reaching vital organs and causing irreversible damages (Section 2). Although various amphibian species are known to prey on Argentine ants (Alvarez-Blanco *et al.* 2017 = Section 1; Beard 2007; Ito *et al.* 2009; Wallis *et al.* 2016), the toxicity of their consumption has never been explored, and could very likely compromise their fitness.

Here, we experimentally measured the survival and growth rates of two terrestrial amphibian species when fed on either the invasive Argentine ant or a native ant, *Tapinoma* cf. *nigerrimum*. We chose two amphibians that are known to consume Argentine ants with different degrees of diet specialization, natterjack toads (*Epidalea calamita*) and western spadefoot toads (*Pelobates cultripes*). We focus on newly metamorphosed toadlets because size variation at metamorphosis is the variable that best predicts juvenile survival to adulthood (Cabrera-Guzmán *et al.* 2013; Gomez-Mestre and Tejedo 2003) and is likely to be the life stage at greater risk from detrimental interactions with invasive ants. Because of the lack of coevolution among native predators and invasive prey, we expected that feeding and coexisting with the Argentine ant could reduce predators' survivorship and/or growth rate, due to higher energy consumption required to detect, capture and digest the new prey, envenomation through the skin, internal damage to vital organs, or a combination of these.

Fig. 5. Newly metamorphosed *Epidalea calamita* are specially vulnerable and their development is crucial in this early terrestrial stage (*Illustration : María León*).

Section 4: The physiological consequences of growing in Argentine ant invaded areas

Despite the ubiquity of birds and ants, their ecological relevance and the effort devoted in studying independently several aspects of their biology, the nature of the interactions between both taxa remains poorly understood. Direct interactions such as predation-prey relationships are potentially reciprocal (Ridlehuber 1982; Stake and Cimprich 2003; Stoddard 1931; Strong 2000; Wiebe and Gow 2013). However, evidence on ant species actively preying upon alive birds is scarce, and has proven difficult to detect than *vice versa*. Despite the differences in size, competition for resources between birds and ants has also been reported through both, exploitative and interference competition (Aho *et al.* 1997, 1999; Haemig 1996; Lambrechts and Schatz 2014; Philpott *et al.* 2005). From these studies, ants seem to win in both types of competition, partially supporting Persson's hypothesis (1985). Persson's hypothesis states that smaller animals should be superior than larger ones in exploitative competition, especially when resources are scarce, but the opposite should be expected in interference competition. Facilitative interactions between birds and ants have also been reported (Haemig 2001), for example when ants take advantage of bird nests because of microclimatic conditions and available food resources (nestling food remains and nest-dwelling fauna) (Mitrus *et al.* 2016). Likewise, birds may follow ant trails (i.e. army ants) to forage on insects flushed by the ant swarm (Di Giacomo and Di Giacomo 2006), or seek ant-protection from other predators by choosing nesting locations selectively (Gilardi and von Kugelgen 1991; Grimes 1973; Janzen 1969; Young *et al.* 1990). Furthermore, interactions may not be mutually exclusive, and be context-dependent by changing from competitive or even predatory to facilitative (Haemig 1999, 2001). Additionally, the intensity of the interactions is time-dependent and varies with different life history stages of the taxa involved (Allen *et al.* 2004; Boieiro *et al.* 2018; Drees 1994; Lambrechts *et al.* 2008). However, although a wide range of interactions between ants and birds have been studied, proximate causes and consequences of most observed interactions remain unclear.

Ant invasions are known to disrupt native ant community and modify the established interactions with other taxa such as birds. There have been several studies on the effect of invasive ants on native birds, particularly during reproduction, which is a sensitive period of their life-history, especially for developing chicks. Most studies report negative effects on both behavior and breeding performance (see reviews in Allen *et al.* 2004; Holway *et al.* 2002; Lach and Hooper-Bui 2010; Suarez *et al.* 2005). Invasive ants have been reported to reduce productivity and induce behavioral shifts by direct attacks or even predation events (eggs, nestlings, adults) (Allen *et al.* 2004; Davis *et al.* 2008; Matsui *et al.* 2009; Plentovich *et al.* 2009; Plentovich *et al.* 2018). Most of these studies concern the red imported fire ant (*Solenopsis invicta*) and also other invasive ants known by their powerful venoms (i.e. *Anoplolepis gracilipes*, *Solenopsis geminata*) (Lach and Hooper-Bui 2010). The invasive Argentine ant is known to compete with native birds over food resources (Estany-Tigerström *et al.* 2010; Rankin *et al.* 2018). However, in most cases the effects on reproduction have been negligible, and seldom compared with the effects of other native ant species (Table 1). Argentine ants' mechanisms of interference with bird reproduction are unknown, and the effects might be subtle and with lagged consequences.

Here, we explored the effects of breeding in Argentine ants' invaded sites in a passerine bird species at its distribution margin, where it is exposed to suboptimal conditions and thus can be particularly sensitive to environmental challenges. The great tit (*Parus major*) is a widely distributed passerine bird species (Gosler 1993). Despite the extensive knowledge on the specie's central population dynamics, information on the demography at the distribution margin is largely incomplete (e.g., Clobert *et al.* 1988; Dhondt *et al.* 1990; Karvonen *et al.* 2012). The limiting conditions of the great tit populations at the distribution margins in southern Europe (i.e. harsh climatic conditions and limited food resources) may bring to light the effects of the Argentine ant on their reproduction, which may be counteracted at optimal condition sites as suggested by Estany-Tigerström *et al.* (2013).

We assessed the nest-site selection and the breeding success of a great tit's population in invaded and uninvaded areas over five years. Moreover, we evaluated the consequences of growing in an area invaded by the Argentine ant by analyzing biometric and several blood parameters related to the oxidative balance and nutritional condition, in both nestlings and adults. We hypothesize that Argentine ants will affect great tit nests and negatively affect their reproduction. To test the Argentine ant's willingness to forage in breeding nests, we performed a field experiment in the study area with artificial nests and eggs. This experiment also allowed us to compare the efficiency in finding different nest contents by invasive and native ants.

Fig. 6. The great tit, Parus major (*Illustration: María León*).

Materials and Methods

Study area

Doñana National Park is located on the southwest coast of Spain, on the right bank of the mouth of the Guadalquivir river (Huelva and Seville, 37° 2'33.24"N, 6°26'4.12"W, from 37°08'32"N, 6°33'46"W to 36°47'41"N, 6°15'07"W, see Fig. 7a). Climate is Mediterranean sub-humid with oceanic influence, and alternates a mild and rainy period in autumn and winter with a hot and dry period in summer. It is a refuge of fauna and flora of 54,252 ha, unique for biodiversity conservation (UNESCO Biosphere Reserve in 1980, Ramsar Wetland of International Importance in 1982, UNESCO World Heritage site in 1994). Doñana wetlands are a key stop on the migratory route of birds, in addition to being a breeding and wintering area. Soils of aeolian origin, together with the proximity of the water-table to the surface, lead to a unique system of more than 3,000 temporary ponds during wet years (Díaz-Paniagua *et al.* 2010; Díaz-Paniagua *et al.* 2016). Doñana ponds and wetlands are home to 11 of the 13 amphibian species present in the southwest of Spain, five of which are Iberian-endemic (Díaz-Paniagua *et al.* 2016; Díaz-Paniagua *et al.* 2005). With respect to ants, more than 40 species live in Doñana, including both Iberian- and local-endemics, which represent 14% of the known species in the Iberian Peninsula (Carpintero 2001; Sánchez-García *et al.* 2017).

The field work in this thesis was carried out at Doñana Biological Reserve (hereafter RBD, 36°59'29"N, 6°27'W, see Fig. 7), which is contained within the National Park. RBD comprises both marshland and a matrix of dry and wet Mediterranean scrublands (Fig. 7a,d). The transitional area between the marshland and the scrubland matrix is called "La Vera". Dry scrublands, or "monte blanco", are characterized by an abundance of *Halimium halimifolium* and rock rose (*Cistus salvifolius*, *C. libanotis*), and in the drier areas, topped lavender (*Lavandula stoechas*), rosemary (*Rosmarinus officinalis*), and thyme (*Thymus mastichina*), alternating with grassland patches. Wet scrublands, or "monte negro", are thicker and grow on very humid soils. The dominant species are heathers (*Erica ciliaris*, *E. scoparia*, *E. umbellata*, *Calluna vulgaris*) mixed with common myrtle (*Myrtus communis*), gorse (*Ulex minor*), wild blackberry (*Rubus ulmifolius*), and bracken (*Pteridium aquilinum*), among others. "La Vera" is an ecotone strip of eutrophic grassland and phreatophytic scrub vegetation (including *Juncus* spp., *Ulex* sp., and *Rubus* spp.), cork oaks, ash (*Fraxinus angustifolia*), and poplar trees (*Populus alba*, *P. nigra*). Scrublands contain isolated cork oak trees (*Quercus suber*) and scattered pine plantations (*Pinus pinea*). The cork oak is a keystone species because it shelters many species against the region's hot, dry summers—the tree's canopy provides shade and the root system keeps shallower soil humid (Kurz-Besson *et al.* 2006)—and its location near temporary ponds results in a clear environmental gradient under the tree canopy (wetter conditions closer to the pond side and drier conditions on the opposite side of the tree) (Fig. 7b,c).

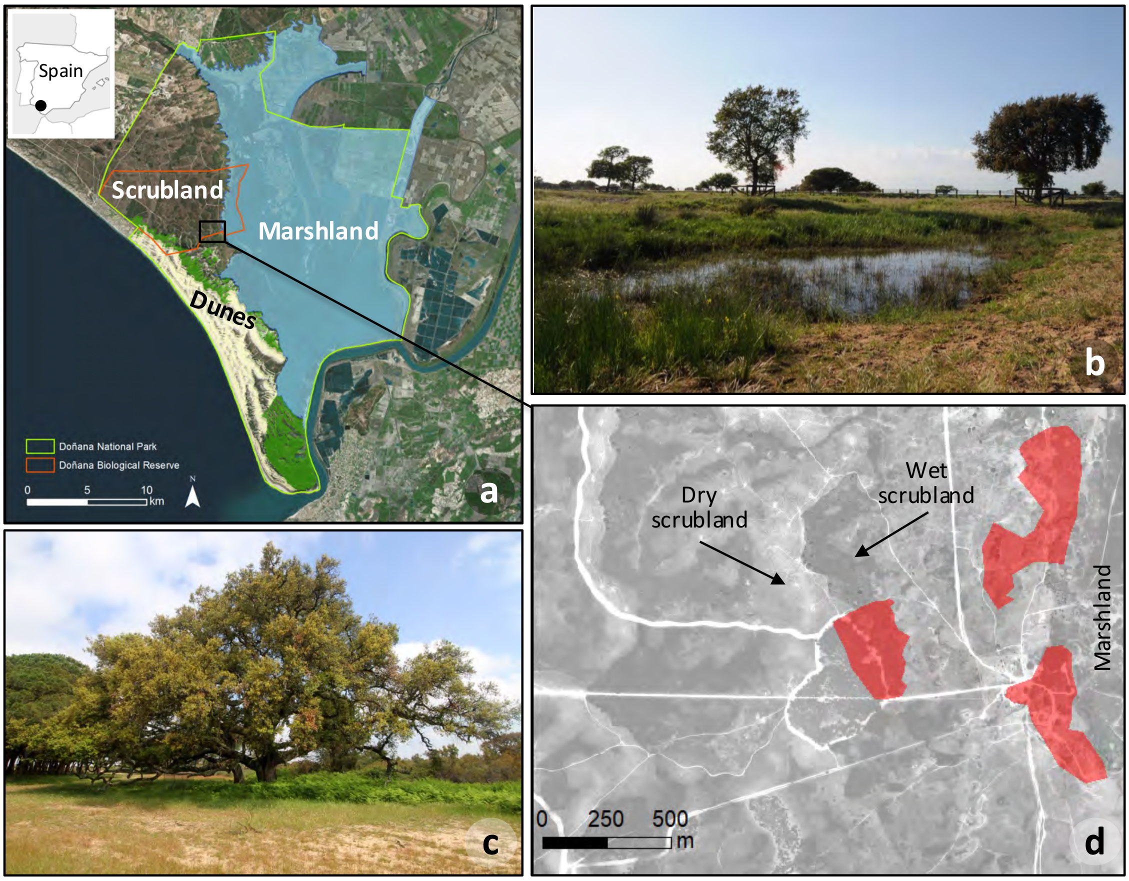


Fig. 7. Study area at Doñana National Park. Location and boundaries of the National Park and the Biological Reserve (a). Argentine ant invasion (red) in the study area (d). Temporal pond (b) and centenarian cork oak tree (c). (*Photo credits: Paloma Alvarez-Blanco (b), Fernando Amor (c)*).

Study species

Native ants

Three native ant species, all omnivorous and commonly found in RBD, were used to carry out comparisons with the invasive Argentine ant: *Tapinoma* cf. *nigerrimum* (Nylander, 1856), *Aphaenogaster senilis* Mayr, 1853 and *Crematogaster scutellaris* (Olivier, 1792) (Fig. 8).

Fig. 8. Native ants *Tapinoma* cf. *nigerrimum* (a), *Aphaenogaster senilis* (b), and *Crematogaster scutellaris* (c) (*Photo credits: Fernando Amor*).

In the study area, *Tapinoma* is the only native genus in the subfamily Dolichoderine, which also includes the invasive *Linepithema* (Carpintero 2001). Thus, *T.* cf. *nigerrimum* is taxonomically related to the Argentine ant, with which it also shares many life-history traits, including polygyny (more than one queen per colony), polydomy (more than one nest per colony), and their main food resource, the honeydew of scale insects and aphids (Arnan *et al.* 2012). *T.* cf. *nigerrimum* is a black polymorphic ant that measures 4 mm on average, and is famous for its peculiar odor of butyric acid (similar to butter). *A. senilis* is an Iberian endemic (Angulo *et al.* 2011; Collingwood and Yarrow 1969) and one of the most abundant scrubland species in the study area (Angulo *et al.* 2011; Carpintero 2001). It is black, monogynous, monomorphic, and three times larger than the Argentine ant (Arnan *et al.* 2012). Finally, *C. scutellaris* is an arboreal two-toned (black and red) ant, also monogynous and monomorphic, and the most abundant species in Doñana's cork oaks, where they live in the tree trunks (Angulo *et al.* 2011; Carpintero *et al.* 2005).

Amphibians

Three of the most abundant terrestrial species in Doñana were selected to perform experimental approaches to evaluate the impact of the invasive Argentine ant on native amphibians (Sections 1-3): the natterjack toad, *Epidalea calamita* (Laurenti, 1768) (= *Bufo calamita*), the western spadefoot toad, *Pelobates cultripes* (Cuvier, 1829) and the Mediterranean tree frog, *Hyla meridionalis* (Boettger 1874) (Díaz-Paniagua *et al.* 2006).

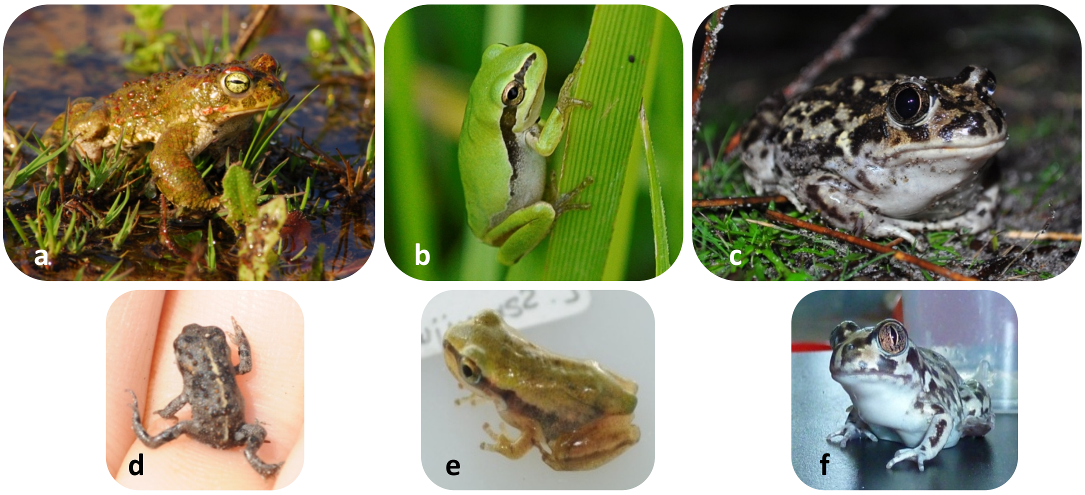


Fig. 9. Adults (a-c) and juveniles (d-f) of the natterjack toad (*Epidalea calamita*), the spadefoot toad (*Pelobates cultripes*) and the Mediterranean tree frog (*Hyla meridionalis*) (*Photo credits: Francisco J. Gómez-Chicano (a), Eduardo Rodríguez-Expósito (b,c), Paloma Alvarez-Blanco (d,e), Carmen Díaz-Panigua (f)*).

The natterjack toad, *E. calamita*, is an active-search hunter and has been commonly observed feeding on ants directly from the entrance of the nest (González de la Vega 1988). It breeds in small, shallow ponds (short hydroperiod in temporary ponds at Doñana) and has a short larval development period (Díaz-Paniagua *et al.* 2010). Newly metamorphosed individuals are very small, and spend about one month feeding in the vicinity of the ponds before dispersing (Díaz-Paniagua *et al.* 2005).

The spadefoot toad, *P. cultripes*, is a more generalist predator, as demonstrated by its “sit-and-wait” foraging strategy (Garcia-Paris 1990). It is highly terrestrial, except in the breeding season, and lives in sandy substrates which it can excavate easily using the characteristic coriaceous spades on its hind limbs (Díaz-Paniagua *et al.* 2005; Recuero 2014). It breeds in deep ponds (long hydroperiod in temporary ponds at Doñana) and has a long larval development period (Díaz-Paniagua *et al.* 2010). Newly metamorphosed individuals may vary greatly in size, but are always much larger than those of *E. calamita*.

The Mediterranean tree frog, *H. meridionalis*, is mainly arboreal and displays an intermediate degree of ant-specialization between *E. calamita* and *P. cultripes*. It breeds in medium to deep ponds (medium and long hydroperiod at temporary ponds at Doñana), and is especially found in ponds next to cork oak trees (Díaz-Paniagua *et al.* 2005). Newly metamorphosed individuals are intermediate in size between *E. calamita* and *P. cultripes*.

The great tit

The great tit, *Parus major* (Linnaeus, 1758), is a widely distributed passerine that occupies a varied range of habitats, from dense deciduous and conifer forests to areas with dispersed trees (Clobert *et al.* 1988). It reaches the southern end of its European distribution in the study region (Shirihai and Svensson 2018). Great tits become territorial during the breeding season, usually keep their mates for life, and breed in the same area in subsequent years (Clobert *et al.* 1988). Great tits nest in tree cavities, which make this species suited to living in artificial nest boxes in which its reproduction can be monitored (Clobert *et al.* 1988).

Fig. 10. Reproductive cycle of the great tit, *Parus major* (*Photo credits: Paloma Alvarez-Blanco*).

The great tit demonstrates sexual dimorphism as well as task division among the sexes. Males defend the territory while females build the nest and incubate the eggs, and both sexes feed the chicks (Clobert *et al.* 1988). Females lay between four to twelve eggs per clutch and start to incubate when the clutch is complete (Clobert *et al.* 1988). Chicks are altricial: they hatch blind, naked, and unable to regulate their body temperature. Parental care in great tits is essential, including brooding, feeding, protection, and nest-sanitation. Adults and offspring have different diet requirements. Adults are mainly insectivorous in spring and summer, and feed on a wide range of seeds in fall and winter, when insect availability decreases (Clobert *et al.* 1988). Chicks demand large amounts of protein per day, with Lepidoptera larvae (caterpillars) as their main food resource (Clobert *et al.* 1988). Chicks fledge after 19-21 days in the nest (Clobert *et al.* 1988). Great tit was used as study model in Section 4.

Ethical issues

Experimental procedures were approved by the CSIC Ethics Committee and complied with Spanish legislation regarding the protection of wildlife used for scientific purposes (*CEBA-EBD 11/36* [Sections 1, 2, 4]; *CEBA-EBD 11/36b* [Section 2]; *CEBA-EBD 529/2016*). The regional government of Andalucía approved the necessary field work (*DGPAG 17/01/2018/004* [Section 3]; *1043/MDCG/mect* [Sections 1 – 4]; *2014-1073-00000613-FQH/MDCG/mect* [Sections 2, 4]; *2015-1073-00001494-FQH/MDCG/mect*, and *2015-1073-00003362-JPCD/MDCG/mes* [Section 4]). Alejandro Bertó-Moran was the veterinarian in charge of animal health and welfare for the Doñana Biological Station (EBD – CSIC) and RBD experimental facilities. Personal authorization to carry out animal experimentation was given by the Spanish MAGRAMA (CAP-T-0220-15 and EXP-000261 to Paloma Alvarez-Blanco, and CAP-T-0224-15 to Elena Angulo).

Distribution of the Argentine ant in Doñana

The Argentine ant is the only introduced ant species in Doñana (Angulo *et al.* 2007; Angulo *et al.* 2016; Carpintero 2001) and virtually the only ant species in the invaded areas (Angulo *et al.* 2007; Angulo *et al.* 2011; Carpintero *et al.* 2007; Carpintero and Reyes-López 2008; Carpintero *et al.* 2003).

In the 1970s, the Argentine ant, *Linepithema humile*, was unintentionally introduced to the RBD in the reserve's field station (Angulo *et al.* 2011). It has spread to nearby natural habitats that have suitable microclimatic conditions (Angulo *et al.* 2011; Carpintero *et al.* 2003, Fig. 7d). Given that queens are wingless and workers travel only short distances (Heller *et al.* 2008), the invasion of natural areas relies on inadvertent and sporadic transport by humans, predators, or scavengers (Carpintero *et al.* 2005, Castro-Cobo et al. *In prep.*). Harsh climatic conditions during summer are the main factor that limits the spread of the invasive ant throughout the area. Thus, dry scrubland presents more of a challenge to the Argentine ant, to the benefit of the more-adapted native ant species (Angulo *et al.* 2011). However, centenarian cork oak trees in Doñana are a perfect microhabitat: they maintain humidity even during summer drought due to their deep roots (Kurz-Besson *et al.* 2006), and Argentine ants take advantage of this. Under the cork oak canopies, a dense network of interconnected nests can be found, and ants also forage in the tree trunk and branches (Carpintero *et al.* 2005). Some pines (*Pinus pinea*) in the northern part of "El pinar de San Agustín", and the poplar trees (*Populus sp.*) nearby the marshland are also invaded (Angulo *et al.* 2011). Invaded and uninvaded trees are quite interspersed. In fact, the Argentine ant is patchily distributed in the study area, which allows a comparison of invaded and uninvaded areas with similar environmental conditions (Fig. 7d).

Section 1: Native predators living in invaded areas: responses of terrestrial amphibian species to an Argentine ant invasion

1.1. Field study

One week of sampling was conducted at RBD during the summer and fall of 2009 and the winter and spring of 2010. Sampling took place in and under ten centenarian cork oaks (hereafter, tree areas), five of which had been invaded and five of which remained uninvaded by the Argentine ant. Each tree area was treated as an independent replicate. To be more certain that the amphibians studied were not experiencing both invaded and uninvaded areas, no invaded tree area was closer than 250 m to any uninvaded tree area. Within groups (invaded or uninvaded), tree areas were separated by at least 40 m. This distance guaranteed independence in ground and tree arthropod sampling (Angulo *et al.* 2007; Gove *et al.* 2009). We were only able to sample a limited number of trees because the National Park restricted amphibian trapping and the access to some invaded trees (because of waterbird conservation concerns) and because the Argentine ant invasion pattern is patchy (Fig. 7d).

Sampling the abundance of amphibian predators

Amphibians were captured using three pitfall trap lines composed of three bucket traps each (30 × 40 cm) and a barrier of 3 m × 50 cm to guide individuals into the buckets (Fig. 11). Traps were deployed for 7 days during each season and checked every 3 h. Most of the individuals captured were euthanized to examine their gut contents and to collect tissue samples for the stable isotope analyses. Samples were kept in 70% alcohol until further analyses could take place in the laboratory.

Sampling the availability of invertebrate prey

Invertebrates were sampled using seven pitfall traps (200-ml PVC cups 2/3 full of soapy water) and two white traps (for flying arthropods; 25 × 50 × 10 cm trays partially filled with soapy water) placed on the ground under the tree canopy (Fig. 11). Another seven pitfall traps were attached to the tree's branches and trunk. Traps were deployed for 3 days per sampling period and the invertebrates, collected every day, were kept in 70% alcohol. All invertebrates were identified to the lowest taxonomic level possible. The total number of individuals was then calculated for each taxonomic group caught for each sampling day, tree area, and sampling season. This number was used to estimate biomass: the number of individuals was multiplied by the mean mass for each taxonomic group, which was obtained by measuring the dry mass of 10–30 individuals.

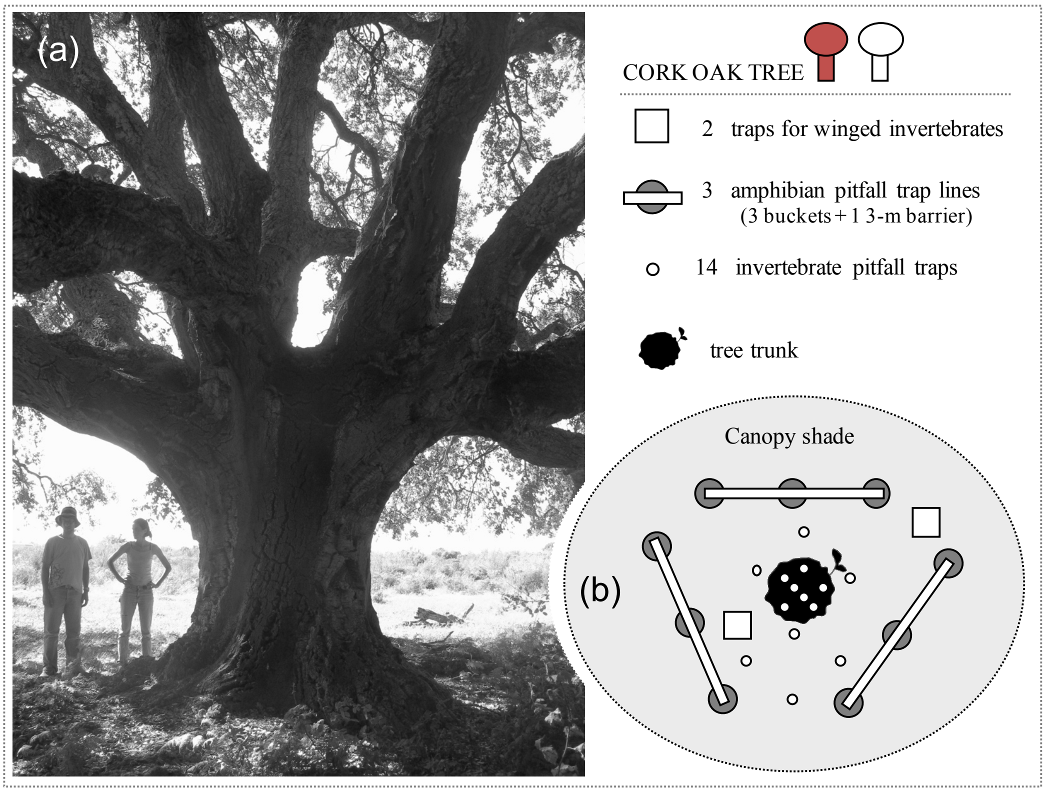


Fig. 11. (a) A picture of a cork oak (*Quercus suber*) and (b) schematic of the trapping protocol for a given tree. The pitfall traps shown inside the tree trunk were attached to the trunk and branches (*Photo credits: Xim Cerdá*).

Stomach content analyses

Predator stomach fullness (i.e., whether the stomach contained food or was empty) was determined, and the stomach contents were removed and preserved in alcohol (70%) until the prey species could be identified to the lowest taxonomic level possible using the invertebrates obtained in the pitfall traps as references. The relative importance of each prey item in a predator's diet was assessed in two ways: (1) using prey biomass: the percentage of total biomass attributable to each prey item (100 × [biomass of a specific prey item/total biomass of all prey items]) and (2) prey frequency: the percentage of each prey item across all non-empty stomachs

(100 × [number of stomachs containing a specific prey item/total number of stomachs containing prey]). To limit bias due to digestion in the biomass calculations, the mass of the whole body of one individual was used, which was determined using the individuals obtained via pitfall trapping.

A cumulative prey curve was constructed to assess whether an adequate number of stomachs had been sampled. The order of the stomachs was randomized ten times, and the mean (± SE) of singleton prey items was plotted to minimize the possible bias resulting from sampling order. The point at which the prey curve approached an asymptote revealed the number of stomachs needed to accurately characterize the diet (Fig. 14).

Isotopic analyses

Stable isotope methods are currently among the most powerful tools used in the study of trophic relationships and animal diets. However, it is difficult to obtain exact estimates of isotopic values, as they can be affected by a number of factors (Caut *et al.* 2009; Post 2002; Vanderklift and Ponsard 2003). To reduce variability when comparing the isotopic values of amphibians captured in different areas and on different dates, it is necessary to obtain an isotopic baseline (Lorrain *et al.* 2015). Isotopic baselines are known to vary across time and space and can influence the range of nitrogen isotopic values within a given food chain at a given time. To estimate the nitrogen isotopic baseline, samples of the most abundant plant species found in the shade cast by the canopy of each tree were collected during each season and identified. The mean of their isotopic values was used as the baseline. To estimate the nitrogen isotopic values for the amphibians, liver samples were collected. Both sample types (plants and amphibian livers) were dried at 60 °C for 48 h, ground to a fine powder, weighed in tin capsules, and stored in a desiccator until isotopic analyses took place. The analyses were performed using a continuous flow isotope ratio mass spectrometry system that consisted of a Flash HT Plus elemental analyzer coupled to a Delta-V Advantage isotope ratio mass spectrometer via a CONFLO IV interface (Thermo Fisher Scientific, Bremen, Germany). The system was located in the Stable Isotope Laboratory at the Doñana Biological Station (LIE-EBD; http://www.ebd.csic.es/lie/Home.html). Isotopic ratios are presented as δ values (‰); they are relative to atmospheric nitrogen and expressed as $\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000$, where R is $^{15}N/^{14}N$. The reference material was IAEA-N1 (+ 0.4‰). Replicates of internal laboratory standards (which had been previously calibrated with international standards such as IAEA-N1) were regularly included in the sampling sequence and indicated that analytical measurement errors never exceeded ± 0.2‰.

1.2. Laboratory preference experiment

Fourteen adult natterjack toads (*E. calamita*) were captured in the field in spring 2013. Eight came from uninvaded areas, and six came from invaded areas. All the adults collected in uninvaded areas were captured 1.5 km away from invaded areas. It is highly unlikely that they had previous contact with the Argentine ant. In the laboratory, they were individually housed and fed mealworms, pillbugs, and small crickets *ad libitum*. The day before each preference trial, the toads were not fed. In addition, several hundred workers were collected from colonies of the Argentine ant and from colonies of three native ant species: *Tapinoma* cf. *nigerrimum*, *Aphaenogaster senilis*, and *Crematogaster scutellaris*.

Two kinds of preference tests were performed. In the first test, five workers of each ant species (for a total of 20 ants) were simultaneously added to a terrarium. A toad was then placed in the center of the terrarium, and the time at which it ate each of the ants over a 30-min period was recorded. Each toad was tested five times (N = 70 trials; 14 individuals; 1,400 ants tested); trials took place at least 3 h apart. In the second test, which also lasted 30 min, 20 ants of the same species were placed in a terrarium, and the time at which the toad ate the ants was recorded. Each toad was tested four times, with each of the four ant species (N = 56 trials; 14 individuals; 1,200 ants tested). The order in which they experienced the species was random, and trials were separated by at least 18 h. Both types of tests were performed because, in the field, *L. humile* rarely co-occurs with native ants. As a consequence, amphibians will rarely have to choose between native and invasive species. However, it is nonetheless informative to quantify preferences and consumption rates under both sets of conditions.

1.3. Statistical analyses

General linear models were used to compare

(a) The number of individuals (dependent variable) of different amphibian species found in invaded versus uninvaded areas across different seasons (independent variables). When juveniles were also found, adults and juveniles were placed in two distinct categories in the "species" variable. This differentiation between adults and juveniles reflects an important spatial constraint related to amphibian biology. Juveniles are unable to choose the ponds from which they emerge, while adults can choose where they forage and breed. The model examining overall amphibian abundance included tree area (five levels), invasion status (invaded vs. uninvaded), season (four levels), and amphibian species [six levels: *E. calamita* (adults and juveniles), *P. cultripes*, *H. meridionalis*, and *D. galganoi* (adults and juveniles)]. We carried out separate analyses for each amphibian species (by specifying the "by" option in PROC GENMOD, SAS Institute Inc. 2008).

(b) Total available biomass and the percentage of available ant biomass (dependent variables) in invaded versus uninvaded areas across seasons (independent variables); the invasion-by-season interaction was also included. The models included tree area (five levels), invasion status (invaded vs. uninvaded), season (four levels), and sampling day (three levels). Thus, the totals used were for each day of each season and for each of the invaded and uninvaded tree areas.

(c) Total biomass in stomach contents and the percentage of ant biomass in stomach contents (dependent variables) for different amphibian species in invaded versus uninvaded areas across seasons (independent variables) (note: *D. galganoi* was excluded from these analyses because of its small sample size). In this case, the sample size was the number of individual amphibians for which stomach contents could be analyzed and were not empty (N = 95, Table 3a). The model included amphibian species (four levels), tree area (five levels), invasion status (invaded vs. uninvaded), and season (four levels). Thus, the totals used were for each amphibian species, for each season, and for each of the invaded and uninvaded tree areas.

(d) The nitrogen isotopic baseline ($\delta^{15}N_{TREE}$ = mean $\delta^{15}N$ of plants; dependent variable) for the different tree areas across seasons (independent variables). The model examining the baseline isotopic values included tree area (five levels) and season (four levels).

(e) The nitrogen isotopic values of amphibians (δ^{15}N of liver tissue; dependent variable) of different species in invaded versus uninvaded areas across seasons (independent variables). To standardize the comparisons, amphibian tissue δ^{15}N values were corrected using the isotopic baseline of each tree during each sampling period ($\delta^{15}N_{COR} = \delta^{15}N_{LIVER} - \delta^{15}N_{TREE}$). In this case, the sample size was the number of individual amphibians for which we obtained isotopic values (N = 106, Table 3a). The model included amphibian species (six levels), invasion status (invaded vs. uninvaded), the invasion-status-by-season interaction, and season (four levels).

As necessary, tree area identity was included as a repeated measures factor ("repeated subject" command in PROC GENMOD, SAS Institute Inc. 2008). Models of the total stomach content biomass also included the sex of the animal as a fixed effect. The normality of all the dependent variables was tested before models were fitted. A Poisson distribution and a log-link function were used for the models of available biomass and stomach content biomass (except in the case of the total biomass of stomach contents, for which a gamma distribution with a log-link function was used); model deviance was, thus, minimized. Because one of the invaded trees was flooded during the winter and the spring, the number of invaded trees was reduced to four.

The data from the preference experiments were analyzed using survival analyses, which estimated the probability of ants being eaten as a function of time. Mixed effects Cox models were used so that random factors could be included; we employed the R package coxme (Therneau 2015, R Core Team 2016). For the first test, the model included (a) ant species, to evaluate toad preference for different prey species; (b) the invasion status of the areas where the amphibians were captured to test for differences in amphibian naïveté to the Argentine ant; (c) the trial order for a given individual, to test whether learning occurred following exposure to the ants; and (d) the ant-species-by-trial-order interaction to test for differences in amphibian learning among ant species. Because learning was ant-species dependent, trial order was also tested for each ant species separately. Individual amphibian identity and trial number were included as random factors. For the second test, the model included only ant species and individual amphibian identity (as a random factor). The significance of each variable was tested using a $\chi 2$ test that compared the likelihood of the full model with that of the full model minus the variable of interest. In the latter model, the interaction between two variables was also removed when the significance of only one of the two variables was being tested. When significant, the model with the highest likelihood value (or the simplest model in case this value was equal) was considered the best.

Section 2: Are amphibians threatened by a global invasive ant?

2.1. Spatial and temporal overlap in ant and juvenile amphibian activity at the local scale

We characterized the activity of ants and newly metamorphosed juvenile amphibians in the field at RBD. In the spring of 2013, during the period when newly metamorphosed *E. calamita* were emerging from ponds, we established two plots that were separated by 400 m. One encompassed two invaded ponds (~15 and 25 m long, respectively), and the other comprised one uninvaded pond (53 m long). For two consecutive days in April and May, we set out baits to characterize ant activity (a 35-m transect with water-diluted honey and cookie baits placed on 10 pairs of plastic spoons), and we conducted amphibian surveys (1-m-wide, 50-m-long transect) throughout the day (at 9:00, 12:30, 16:00, 19:30, and 23:00). The baiting and surveys were carried out at the same locations to assess spatial overlap between ants (native or invasive) and amphibians. We recorded the number and species of ants and toadlets during each sampling session.

In May and June 2013, 2014, and 2018, which is the emergence period of juvenile amphibians, particularly *H. meridionalis* and *P. cultripes*, we counted the number of dead juveniles found along 40 m of *L. humile* ant trails. We searched in a band of 40 cm from the trail. A number of trails were used. They are relatively permanent, have been active for many years, and are located near a pond and a laboratory building. Counts were carried out over four days during each period.

2.2. Housing of experimental animals

Juvenile amphibians were assigned to four different experiments: the ant-trail-exposure experiment (2.3), carried out in the field at RBD; the foraging-arena-exposure experiment (2.4), carried out in experimental facilities at RBD, where temperature and photoperiod conditions were similar to those in the field; and the iridomyrmecin-exposure experiment (2.5) and the dose-response experiment (2.6), both carried out in experimental facilities at EBD under controlled conditions (23 ºC, 12:12 photoperiod, 60% humidity). In the first two experiments, juveniles were released near their ponds of origin 48 h after the tests. In the last two experiments, juveniles were euthanized using an overdose of anesthetic (5-min bath in tricaine methasulfonate [MS-222], 10 g/L dissolved in Ringer's lactate solution). In the iridomyrmecin-exposure experiment, euthanasia occurred 48 h after the test. In the dose-response experiment, it occurred approximately 10 min after dose application, right after the clinical evaluation (because these individuals were used for histological and chemical analysis).

We collected juvenile amphibians in the field near ponds shortly after emergence. We also collected tadpoles that were raised until they reached metamorphosis. They were kept in an experimental facility, either at RBD (raised in 55 L tanks, fed common aquatic plants, ambient temperature and photoperiod) or EBD (raised in 5 L plastic containers, fed rabbit chow *ad libitum*, 23 ºC, 12:12 photoperiod). Juveniles were housed in groups (up to 10 individuals from the same pond of origin) in 20 x 30 x 20 cm terrariums (with sandy substrate, cork pieces as shelter, and a water container [in the case of *H. meridionalis*]) that were cleaned weekly. Every two days, we checked on the juveniles, misted the terrariums with water, and gave individuals *ad libitum* mealworms, *Drosophila* flies, and small crickets dusted with a calcium supplement. During the experimental trials, juveniles were maintained individually in smaller containers.

Each individual was only used once. Sampling/capture order determined the allocation of individuals to experimental groups: each new individual was assigned to a treatment on a rotating basis (i.e., treatments were alternated). Individuals or individual samples (such as tissues) were identified with a code; researchers were thus blind to treatment assignments when conducting analyses (e.g., histological or chemical analyses). Because these experiments were being carried out for the first time, we had no estimates of variation for the dependent variables (i.e., the effect of the Argentine ant on juvenile amphibians), which prevented us from using power analysis to calculate a minimum sample size. Consequently, sample size was chosen as follows to comply with ethical guidelines—we sought to limit the number of individuals used while ensuring that we had adequate statistical power given the numbers and types of variables in each planned analysis. In some cases, sample sizes were unbalanced because the availability of amphibian species in the field varied. The total number of individuals used was as follows: 185 *P. cultripes* (30 for the ant-trail-exposure experiment, 94 for the foraging-arena-exposure experiment, 42 for the iridomyrmecin-exposure experiment, and 19 for the dose-response experiment); 137 *H. meridionalis* (27 for the ant-trail-exposure experiment, 75 for the foraging-arena-exposure experiment, and 35 for the dose-response experiment); and 152 *E. calamita* (125 for the foraging-arena-exposure experiment and 27 for the dose-response experiment).

Two native ant species, *Tapinoma* cf. *nigerrimum* and *Aphaenogaster senilis*, were collected at RBD and used to carry out comparisons with *L. humile*. Five fragments of *L. humile*, *T.* cf. *nigerrimum*, and *A. senilis* colonies were maintained at RBD for the foraging-arena-exposure experiment (2.4). They were housed in dark, enclosed nesting areas (10 cm in diameter; height of 10 cm for *A. senilis* and 5 cm for *T.* cf. *nigerrimum* and *L. humile*). These nesting areas were connected to open foraging arenas (30 x 10 x 10 cm), where ants were fed using a small Petri dish permanently located in the arena. Another five fragments of *L. humile* and *T.* cf. *nigerrimum* colonies were maintained at EBD. They were housed in open containers (30 x 10 x 10 cm) with a dark-colored tube acting as a nest. These fragments were used for the dose-response experiment (2.6) and to carry out histological and chemical comparisons between the ant species (2.8). All ants were fed *ad libitum* fresh fruit, mealworms, and diluted honey.

2.3. Ant-trail-exposure experiment

In the field, we searched for ant trails belonging to *L. humile*, *A. senilis*, and *T* cf. *nigerrimum*. Although the two native ants do not set up permanent trails, they can use trails as a foraging strategy (Cerdá *et al.* 2009; van Oudenhove *et al.* 2012). We carefully positioned 10 *P. cultripes* and 10 *H. meridionalis* juveniles 3 cm away from trails of the three ant species. Each amphibian was kept in place using an inverted plastic Petri dish (5.5 cm in diameter, 1.4 cm in height). The sides of the dish were perforated with eight to ten holes large enough to allow ants to enter. The amphibian could move and turn around but not escape. Because the native ant *A. senilis* is larger, tests with this species used a cage (8 x 8.5 x 3 cm, with a mesh width of 5 x 5 mm). The dish or cage was held in place by hand, avoiding any disturbance of the ant trail. Ants took time to discover the amphibians. After the first contact with the ants, the amphibians were kept in place for 2 additional minutes and then released (the dish/cage was carefully removed). They were followed for 10 min or until they moved at least 1 m away from the trail (considered as if they would have escaped), whichever came first.

After the experiment, all individuals were kept under observation for 48 h at RBD experimental facilities to evaluate treatment effects. Individuals were classified as follows: dead, if they died during the 48-h observation period; paralyzed, if they showed signs of paralysis by the end of the observation period; alive, if they neither died nor showed signs of paralysis by the end of the observation period. If an individual was first paralyzed but then died, it was classified as “dead”.

Statistical analyses: For each ant-amphibian species pair, we determined whether observed frequencies of alive, paralyzed, and dead juveniles were different from the expected frequencies (all individuals alive, none paralyzed, and none dead) using a non-parametric $\chi 2$ test (StatSoft Inc 2002).

2.4. Foraging-arena-exposure experiment

In the laboratory, juveniles of *P. cultripes*, *E. calamita*, and *H. meridionalis* were introduced in the foraging arenas of laboratory colonies (see 2.2 for ant colony details) of the three ant species, *L. humile*, *T.* cf. *nigerrimum*, and *A. senilis*. A single amphibian was placed in the colony’s foraging arena for a maximum of 10 min. We measured the time to amphibian discovery and the maximum number of ants on amphibians over the course of a 10-min period. Trials were stopped earlier for ethical reasons, including the possibility that an amphibian would suffer irreversible harm. Such situations included amphibians remaining immobile/paralyzed for a minute or being dragged off by ants, risking serious ant-induced injury. No amphibians died during the 10-min trials. After 48 h of observation, individuals were classified as alive, paralyzed, or dead, as in the previous experiment.

Statistical analyses: We evaluated differences in how *L. humile*, *A. senilis*, and *T.* cf. *nigerrimum* behaved towards juvenile amphibians placed in their foraging arenas. The data (time to amphibian discovery and the maximum number of ants on amphibians) were analyzed using generalized linear models employing a gamma distribution and a Poisson distribution, respectively, and a logit link function (PROC GENMOD, SAS Institute Inc. 2008). Ant species and amphibian species were fixed independent variables; the number of ants in the foraging arena at the beginning of the trial and amphibian mass were covariates (the latter was only used in the model where the maximum number of ants on the amphibian was the dependent variable). When the results were significant, we performed post-hoc comparisons among ant species (using the "contrast" command in PROC GENMOD, SAS Institute Inc. 2008).

As for the previous experiment, we used a non-parametric $\chi 2$ test to compare observed and expected frequencies for each ant-amphibian species pair.

2.5. Iridomyrmecin-exposure experiment

Toadlets of *P. cultripes* were assigned to one of four groups: the control group or one of the three treatment groups, which experienced different doses of iridomyrmecin. There were 10 individuals in each treatment group. In all four groups, the solutions (pure hexane or hexane + iridomyrmecin) were applied to cavity slides, and the solvent was allowed to evaporate. The toadlets were then rubbed on the back with the slides. Hexane solvent was used for the control group. In the treatment groups, different doses of iridomyrmecin, dissolved in hexane, were used: 0.1 mg per toadlet; 1 mg per toadlet; and 5 mg per toadlet. After 48 h of observation, individuals were classified as alive, paralyzed, or dead, as in the previous experiments.

Statistical analyses: Because no individuals died in this experiment, we analyzed the proportion of those who remained alive versus paralyzed using a generalized linear model employing a binomial distribution and a logit link function (PROC GENMOD, SAS Institute Inc. 2008). When there was a significant treatment effect, we performed planned post-hoc comparisons (using the "contrast" command in PROC GENMOD, SAS Institute Inc. 2008), which compared the control group to each of the treatment groups.

2.6. Dose-response experiment

To assess the number of ants necessary to elicit an effect, we constructed dose-response curves for each ant species and each amphibian species. Each amphibian received a single dose and was clinically evaluated 10 min later. Depending on the effect (and on the effects observed in previously tested individuals), a higher or lower dose was applied to the next individual. The mass of each individual was known before the test, and the ant dose (number of ants/g of juvenile) was calculated in order to fill in the gaps in the dose-response curve. Each dose was obtained from a given number of mashed ants (*L. humile* or *T* cf. *nigerrimum* workers) and was prepared in a ceramic bowl using 0.2 ml of dechlorinated water. We used whole mashed ants instead of pygidial gland contents to avoid the loss of iridomyrmecin, which is highly volatile, during gland dissection. The mash was then immediately applied to the back of an amphibian. Then, the individual was placed back in its box for 10 minutes. After this time, all individuals were gently bathed in dechlorinated water to remove the mash, and we then conducted the clinical evaluation.

For ethical reasons, minimal numbers of amphibians were used, and ant dosage levels were limited to what was necessary to obtain adequate dose-response curves (11 and 16 *E. calamita*, 14 and 5 *P. cultripes*, and 21 and 14 *H. meridionalis* for the *L. humile* and the *T.* cf. *nigerrimum* curve, respectively). In the clinical evaluation, we categorized each amphibian as affected or unaffected based on neurological damage. We examined several parameters reflective of the functioning of different parts of the neurological system (Kahn 2005). We evaluated (1) a motor response (we extended and released a leg and noted whether retraction occurred) and the nociception response (presence/absence of reaction to pain, determined by the reaction to pressing a toe with tweezers), which reflected effects on the spinal cord; (2) photopupillary reflexes (presence/absence of response to light changes) which reflected the midbrain response (i.e., in the ocular [II] cranial nerve); and (3) palpebral reflexes (whether the eyelid closed when we touched the medial and lateral canthus of the eye), which reflected the response of the medulla oblongata and the pontine nucleus (i.e., in the trigeminal [V] and facial [VII] cranial nerves). An individual was considered to be affected if any abnormal reaction was displayed.

Statistical analyses: The effect on amphibians (affected vs. unaffected) was analyzed using generalized linear models employing a binomial distribution and a logit link function (glm function in the R package stats, R Core Team 2016). Ant number (corrected for amphibian mass) was the independent variable of interest. Ant species and amphibian species were also included as independent variables. Dose-response curves were obtained from models on each ant-amphibian species pair. The toxic dose represented the number of ants per gramme of amphibian expected to elicit a toxic effect (affected). It was calculated using the function dose.p in the R package MASS (Venables and Ripley 2002) from the dose-response curves.

2.7. Physiological effects on juvenile amphibians

To elucidate the venom's modus operandi and confirm that the damage was caused by iridomyrmecin, we euthanized the amphibians used in the previous experiment after the clinical evaluation (see 2.6). Half the amphibians were used to quantify iridomyrmecin levels in tissues: animals were dissected to remove their brains, livers, and kidneys, which were then individually embedded in hexane (hexane extraction, GC-FID, similar to iridomyrmecin quantification in ants, see 2.8). The other half were used in histological analyses: individuals were fixed in formalin and dissected to obtain their livers and kidneys. Tissue samples were embedded in paraffin wax, sectioned at a thickness of 6 μm using a Leica RM 2155 microtome, and mounted on glass slides. For hematoxylin/eosin staining, sections were previously dewaxed through a series of xylene and ethanol washes (from 100% solution to 100% H2O), stained and rehydrated through a series of ethanol washes (from 70% to 100% solution to 100% xylene), and mounted with cover slides using DPX. Acute lesions found in the liver and kidney tissues, which could be due to a toxin, were evaluated under a microscope (Axio Imager, A1, Zeiss; objective EC Plan-NEOFLUAR 20x/0.5, ∞/0.17), focusing on sensitive areas such as the periportal spaces in both the liver and the renal tubules as well as the glomeruli in the kidneys.

Statistical analyses: First, we analyzed whether the clinical response (affected vs. unaffected) was related to the concentration of iridomyrmecin (μg.g-1 of juvenile) in the brain using a generalized linear model employing a binomial distribution and a logit function (glm function in the R package stats, R Core Team 2016); the model took amphibian species into account. Second, we examined the relationship (lm function in the R package stats) between the concentrations of iridomyrmecin (μg.g-1 of juvenile; log transformed) in each tissue and the quantity of iridomyrmecin (μg.g-1 of juvenile) applied to each juvenile, which was estimated based on the species-specific iridomyrmecin contents. Finally, we tested whether higher doses (μg.g-1 of juvenile; log transformed) corresponded to the presence of lesions in amphibian tissues (liver and kidney); a general linear model (PROC GENMOD, SAS Institute Inc. 2008) was used for each tissue in which the identity of the amphibian species was taken into account.

2.8. Histological and chemical differences between the invasive and native Dolichoderinae ants

Species-specific mean fresh mass for *L. humile* and *T.* cf. *nigerrimum* was obtained by weighing 50 live ants per species in groups of 10. To measure species-specific mean dry mass, ants were preserved in alcohol and dried for 48 h at 60 ºC; then, 10 groups of 10 ants were weighed per species.

We used histological analysis to examine the abdominal glands of the two species. Ant gasters were fixed in 2% glutaraldehyde (buffer: 0.05 M Na-cacodylate and 0.15 M saccharose), postfixed in 2% osmium tetroxide, and embedded in Araldite. Semithin sections (thickness of 1 µm) were created with a Leica EM UC6 ultramicrotome and stained with methylene blue and thionin. These sections were then viewed and photographed with an Olympus BX-51 microscope to check for/examine the abdominal glands.

We investigated chemical differences in pygidial gland composition between *L. humile* and *T.* cf. *nigerrimum*. We sampled 15 ants from each colony. We dissected the pygidial glands of five freeze-killed ants immediately after death (ant was gently deposited in an iced glass Petri dish); the other 10 ants were kept whole. All the samples were then placed in hexane. We identified the different chemical compounds present in the pygidial glands of both species via gas chromatography coupled with mass spectrometry (GC-MS), using an HP-5MS capillary column that was programmed to increase in temperature from 60 ºC (1 min hold) to 320 ºC at a rate of 10 ºC.min-1. Then, the whole ants were used to determine the amount of iridomyrmecin per ant; we did not use the pygidial glands because of the risk of content leakage during dissection. Iridomyrmecin levels were analyzed using gas chromatography (GC-FID - Shimadzu 2010 equipped with a 30 m x 0.25 mm i.d.-BPX5, 0.25 mm capillary column). Helium was used as the carrier gas (flow rate of 35.1 ml.min-1). The injection port and detector temperatures were set to 280 ºC and 310 ºC, respectively. The GC oven was programmed to heat at a rate of 10ºC.min-1 from 60 ºC to 300 ºC, with a 1-min initial hold and a 20-min final hold. Decyl-alcohol (99%) was used as the internal standard, and the calibration curve for quantifying iridomyrmecin concentrations in the samples was constructed using synthetic iridomyrmecin (Chauhan and Schmidt 2014). The amount of iridomyrmecin was then quantified by calculating the area under the peak relative to the internal standard for the different samples and was corrected by the calibration curve.

Statistical analyses: We performed a general linear mixed-effects model to test for differences in iridomyrmecin quantities (square root transformed) between *L. humile* and *T.* cf. *nigerimum*; covariance within colonies was included as a random factor. The model was fitted using the function lmer in the R package lme4 (Bates *et al.* 2015).

2.9. Literature review on the functional ecology of iridomyrmecin

We searched the ISI Web of Science for the word “iridomyrmec*” to obtain published articles about iridomyrmecin (accessed 15th November 2018). The search returned 61 articles. We increased this total by finding additional articles cited therein. In each publication, at the first mention of iridomyrmecin, we noted the function of iridomyrmecin as assessed by the authors. We established the following categories for these functions: defense, insecticide, antibiotic, alarm, antibacterial, trail pheromone, cat-attracting chemical, necrophoresis, or no function specified. Publications could fall into more than one category. We also categorized each article with respect to its main subject: synthesis of iridomyrmecin, iridomyrmecin in other species, chemical composition of exocrine secretions, chemical structure, insecticide, trail pheromone, defensive compound, pharmacological research, antibiotic, necrophoresis, or alarm pheromones (Appendix 1). Finally, we analyzed the relative importance of each described function of iridomyrmecin in the literature, and which other species have and use iridomyrmecin and for what purpose.

2.10. Potential global effects on amphibians

Information on Argentine ant locations was obtained from the Global Biodiversity Information Facility (GBIF), AntWeb (AntWeb) and The Global Ants Database (GLAD; Gibb *et al.* 2017) websites; we gathered a total of 1,407 geographic records. Amphibian ranges and IUCN status were obtained from the IUCN Red List (IUCN v.2017-3). We used the function gContains in the R package rgeos (Bivand and Rundel 2017) to extract amphibian species whose distribution polygons overlapped with the ranges of any given ant population. We then filtered this list of species using IUCN habitat categories to exclude amphibian species that did not use similar macrohabitats to *L. humile* (Appendix in Digital CSIC: http://hdl.handle.net/10261/173421).

Ants and amphibians may further be segregated by differences in microhabitat use. We used the eight categories of microhabitat, described in Moen and Wiens (2017), that adult use outside of the breeding period and included species from our dataset, employing habitat descriptions from the IUCN Red List and the AmphibiaWeb database (AmphibiaWeb). We then utilized microhabitat as a filter: we excluded any amphibian species that only occur in aquatic, semi-aquatic, or torrential microhabitats, where *L. humile* would not likely occur (see Appendix in Digital CSIC: http://hdl.handle.net/10261/173421).

Juvenile amphibians are likely to use slightly different microhabitats from adults (Duellman and Trueb 1994; Wells 2010). We therefore considered the full dataset to be the maximum number of possible amphibian species overlapping spatially with the ants and the microhabitat-filtered list to be the minimum. We acknowledge that we are likely overestimating risk, which is not solely determined by spatial overlap. Additionally, the ant's impact will depend on the amphibian species' biological traits, such as anatomy, behavior, or physiology.

From the full dataset, we determined amphibian species richness per ant locality. Then, using both the full and microhabitat-filtered datasets, we calculated cumulative species richness for amphibians co-occurring with ant populations in the following regions (categorized as "regions" based on the continent—or section of continent—associated with the Argentine ant locations): the Argentine ant's native range, the rest of South America (i.e., outside of the Argentine ant's native range), North America (plus one locality in Japan), Europe (including European island colonies in North Africa), Sub-Saharan Africa, and Oceania. The Argentine ant's native range is conservatively delimited by the Paraná river basin. Finally, for each of these regions and for both datasets, we assessed the proportion of amphibian species in the five different IUCN Red List risk categories.

Section 3: Survival and growth of native toadlets feeding on an invasive ant prey

Tadpoles of natterjack toad (*E. calamita*) and western spadefoot toad (*P. cultripes*) were collected at RBD through dip-netting and brought to climatic chambers at EBD, where they were kept in tap water dechlorinated through activated carbon filters. The climatic chambers (Aralab, Fitoclima 5000) were set at a 12:12h L:D cycle, 24 ± 1 ºC, and 60 ± 10% RH. To avoid the potential effects of microclines in temperature in the chambers, toadlets' containers were rotated every two or three days within and among shelves. Tadpoles were raised individually in either 1 L (*E. calamita*) or 3 L (*P. cultripes*) plastic buckets and fed rabbit chow *ad libitum*. Water was changed twice a week.

As tadpoles completed tail resorption (Gosner developmental stage 46, Gosner 1960), toadlets were weighed to the nearest mg for *E. calamita* (Scales Sartorious CPA225D) and to the nearest 0.01 g for *P. cultripes* (Acculab ALC-2100) and kept in lidded square plastic containers (*E. calamita*: 250 mL; *P. cultripes*: 750 mL). To allow the toadlets to bury and to offer a high visual contrast to distinguish the ants, the containers were filled with a layer of white sand. To ensure animal hygiene, the substrate was cleaned of fecal pellets and prey corpses, moistened, mixed and flattened three times per week and renewed every two weeks. Toadlets were provided with UV-B lighting to enable synthesis of calcitriol (Antwis and Browne 2009). Toadlets were fed pinhead crickets (*Acheta domesticus*, hereafter crickets) *ad libitum* dusted with vitamin complex until the experiments began. Crickets were smaller, in their first instar, for *E. calamita* than for *P. cultripes* (4 vs. 37 mg dry mean weight respectively).

The effects of consuming the invasive Argentine ant (*Linepithema humile*) were compared to those of consuming a phylogenetically close native ant, *Tapinoma* cf. *nigerrimum*. Both ants possess iridomyrmecin, a lethal venom when spread over the skin of amphibian toadlets (Section 2). However, only the invasive ant causes the death of the toadlets, because its stock of iridomyrmecin is far greater than in the native ant (Section 2). To feed the toadlets with living ants, fractions of ant colonies of the two species were collected at RBD, brought to the laboratory, and fed *ad libitum* along the experiment with mealworms, small crickets and sugar solution.

3.1. Experiment 1: Raising metamorphic toadlets on invasive or native ants

96 *E. calamita* and 52 *P. cultripes* toadlets were randomly assigned to either one of two treatments: being raised feeding on the invasive Argentine ant or on the native ant *T.* cf. *nigerrimum*. Toadlets' main diet during the experiment was based on an increasing amount of crickets dusted with a vitamin complex every other day (3 times/week). The experimental treatments consisted in weekly supplementation of the main diet of each toadlet with six ants, either the invasive or the native species. Only two living ants were provided simultaneously at any given time to prevent risks of injuries resulting from the ant venom (Section 2). Additional ants were provided as toadlets consumed them up to six. Both crickets and ants were provided alive, and toadlets had to detect and capture them.

Survival and growth

The experiment lasted for 62 days and toadlets were surveyed daily for survival, and weighed at the beginning of the experiment and weekly afterwards to monitor their growth. Food intake was recorded as the proportion of eaten crickets and ants per week.

Foraging efficiency trial

The foraging efficiency of the toadlets was evaluated as a proxy for their general condition by the end of the experiment (following Gómez-Mestre and Tejedo 2005). Once the foraging experiment was over, toadlets were starved for 3 days and the trial was performed on the fourth day (*P. cultripes* at night; *E. calamita* in the afternoon). Trials were performed in the same container where the toadlets were housed to minimize handling. Right before the trial, the substrate was cleaned and flattened to keep the prey from hiding. Toadlets were acclimatized for two minutes prior to the trial. Each individual was offered the same amount and size of crickets as they used to have in the meals from the previous week (*E. calamita*: 15 small crickets in their first instar; *P. cultripes*: 5 medium sized crickets). Trials were filmed during 5 min from the time crickets were added to the arena. For *E. calamita* toadlets the experiment was repeated two weeks later with the same individuals to test results consistence over time due to the high mortality experienced and consequent low sample size. To test the foraging efficiency, we used three variables: time to prey discovery, the number of attempts to prey (motivation), and prey capture success (accuracy). Attempts to capture prey were easy to recognize because *P. cultripes* toadlets jump towards the prey to capture it and *E. calamita* toadlets project and retract the tongue in a noticeable way (Gómez-Mestre and Tejedo 2005).

3.2. Experiment 2: Feeding on invasive or native ants

Because mortality of *E. calamita* toadlets was high in the presence of live ants, we isolated ant consumption with a feeding experiment. We used *E. calamita* toadlets at two months after metamorphosis to assess whether toadlet growth rate was affected by consumption of the Argentine ant. We randomly assigned five *E. calamita* toadlets to each of the three following treatments: feeding on the invasive Argentine ant, feeding on the native ant *T.* cf. *nigerrimum,* and feeding on small crickets in their first instar to control for handling stress. Toadlets were fed ten ants (either invasive or native ants) or eight crickets twice a week for one month. Each feeding day prey items were placed directly on the toadlets' tongue four times to introduce prey items in groups of ants (3+3+3+1) or crickets (2+2+2+2) and we checked that all items were swallowed. We recorded *E. calamita* growth rate weighing the toadlets weekly. Toadlets were fed *ad libitum* with live crickets.

Histology

To assess whether the ingestion of invasive Argentine ants caused internal damages to the organs, we conducted histological analyses of their digestive system. Toadlets were euthanized at the end of the feeding experiment with an overdose of MS-222, and dissected to obtain their stomach and representative parts of their gut (proximal and distal, ileocecal valve). Tissue samples were preserved in 4% formaldehyde, embedded in paraffin wax, sectioned at a thickness of 6 µm using a Leica RM 2155 microtome and mounted on glass slides (longitudinal and transversal sections for stomach and gut, respectively). For hematoxylin/eosin staining, sections were previously dewaxed through a series of xylene and ethanol washes (from 100% to H2O), stained and rehydrated through a series of ethanol washes (from 70% to 100% to xylene), and mounted with cover slides using DPX. Medium-term lesions found in the stomach and gut tissues, which could be due to a toxin, were evaluated under a microscope (Axio Imager, A1, Zeiss. Objective EC Plan-NEOFLUAR 20x/0.5, ∞ /0.17). We quantified these lesions as the presence or absence of pathological aggregated lymphoid infiltrates in the mucosa and submucosa layers including both, focal and diffuse lesions. We also measured the nodule area when focal lesions were found with Image J software to the nearest μm (Schneider *et al.* 2012).

3.3. Statistical analyses

Analyses were performed separately for both toadlet species due to biological differences in their development. All analyses were performed in R version 3.3.3 (R Core Team 2017). Linear mixed models and generalized linear mixed models were fit using the functions lmer and glmer, respectively (package lme4, Bates *et al.* 2015). Linear models and generalized linear models were fit using the funcitons lm and glm, respectively. The significance of the variables to the model was tested using the function drop1. This function compared the model including and excluding the variables based on the likelihood-ratio test statistics (option test = "Chi") except in the linear model, in which it performed a type II ANOVA (option test = "F"). Plots were performed with the package ggplot2 (Wickham 2009).

Experiment 1

Amphibian survival differences among treatments (diet supplemented with invasive or native ants) were analyzed with a Cox proportional hazards regression model using the function coxph in the R package survival (Therneau 2015). In a preliminary analysis we tested the significance of the interaction between toadlet weight at the beginning of the experiment and the treatment. For both species, the interaction was not significant ($\chi^2 = 0.37$, $p = 0.541$, $N = 52$; and $\chi^2 = 0.05$, $p = 0.820$, $N = 96$, for *P. cultripes* and *E. calamita* respectively), indicating that although initial weight influences survival odds, its effect was homogeneous between treatments. Survival analysis was then carried out with treatment as the unique independent factor.

Amphibian growth differences between diet supplemented with invasive ants or native ants were analyzed with a linear mixed model. Toadlet weight (dependent variable) was transformed to satisfy parametric assumptions (log transformation in *P cultripes* and inverse in *E. calamita*). Independent variables were treatment (invasive vs. native ant diet), time (days from the beginning of the experiment) and their interaction. A significant interaction would indicate a difference in growth between treatments, and was therefore the focus of the analysis. Repeated measures were accounted for by including the individual as a random factor. Toadlet weight at the beginning of the experiment was included as a covariate. We only analyzed individuals that survived until the end of the experiment (N = 49 and N = 16 for *P. cultripes* and *E. calamita*, respectively).

Food intake was controlled to know whether the number of eaten prey differed between treatments, which could have affected toadlet growth. Only data corresponding to an entire week was considered. Toadlets were fed an increasing amount of crickets throughout the experiment. ***Cricket intake*** per week in relation to the number of crickets offered was analyzed using a binomial distribution. We used a generalized linear mixed model with repeated measures by including the individual as random factor. We included as independent variables treatment, time, and their interaction. Toadlet weight at the beginning of the experiment was included as covariate (the log or the inverse of the weight was used in order to obtain model convergence for *P. cultripes* and *E. calamita*, respectively).

Ant intake was also controlled to know if it explained toadlet growth. A fixed amount of ants, two each time, six per week, were available for each toadlet throughout the experiment. Weekly ant intake was analyzed with a Poisson distribution. We used a generalized linear mixed model with repeated measures by including the individual as random factor. The treatment, the time and their interaction were considered the main independent variables; if the interaction was not significant it was excluded from the model. Toadlet weight at the beginning of the experiment was included as covariate. Time was transformed (log) in order to obtain model convergence, as well as toadlet weight (inverse) only in *E. calamita* models.

Experiment 2

We evaluated ***E. calamita growth*** among treatments (after being fed manually a supplement of invasive ants, native ants or no ants but crickets, for one month) with a linear mixed model. The dependent variable was toadlet weight. As independent variables we included treatment, time (days from the beginning of the experiment) and the interaction between treatment and time, which was the variable of interest since it represents the differences in growth among treatments. Toadlet weight at the beginning of the experiment was included as covariate and toadlet individual as random factor to account for repeated measures. When the interaction between treatment and time was significant in the model, we further tested for differences in the slopes among the three treatments with a post-hoc Tukey test, using the function lstrends in the R package lsmeans (Lenth 2016).

To evaluate if the intake of the invasive Argentine ant caused ***lesions in the digestive system*** of *E. calamita* toadlets, we analyzed two variables. First, the presence or absence of lesions in the stomach and gut was analyzed with a generalized linear mixed model with binomial error distribution, where the individual was included as random factor. Second, the estimated area of nodules was analyzed with a linear model. Focal lesions were only found in the stomach and consequently the nodules area was only analyzed in this organ. In both analyses, treatment was the fixed factor (with three levels: invasive ant, native ant, and no ants).

Section 4: The physiological consequences of growing in Argentine ant invaded areas

4.1. The Argentine ant attraction to artificial avian nests

To assess the Argentine ant willingness to forage in breeding nests and their use of bird remains, we performed an experiment in June and July 2014 at RBD. The use of artificial nests to evaluate the degree of nest predation has been extensively used in ecological studies. Despite the evidence suggesting that care must be taken when comparing predation on artificial vs. natural nests, still remains as a valid method (Moore and Robinson 2004). We placed artificial nests hanging from a nylon thread tied around the trunk of 10 cork oak trees and 10 pine trees at 1.5 m high. Five of the trees of each species had been invaded and five remained uninvaded by the Argentine ant. Invaded and uninvaded trees were interspersed.

Artificial nests were placed in the North side of the trunk, in order to avoid direct sun exposure and related high temperatures (Ardia *et al.* 2006). Nest structures consisted of a commercial plastic nest in the shape of a grid bowl covered with a commercial cotton layer in which the treatment was added. We used white structures to be able to distinguish properly the number of ants in the nests.

Every three days we alternated randomly one of the five following treatments in each artificial nest: empty, untouched eggs, pierced eggs, feces, and pierced eggs plus feces. In the empty treatment no remains were added. In the untouched eggs treatment we added three quail eggs. Pierced eggs were the same type and number of untouched eggs with three holes (~4 mm diameter) simulating a chick piping the egg-shell during hatching. We used chicken feces (~5 g) added to the cotton layer. Both treatments, empty and untouched eggs were considered control treatments, without interest for the ants living in the study area, since they were not able to break the eggs' shell. Quail eggs were obtained commercially, and poultry feces were obtained from a farmer.

We placed each treatment in the artificial nest structure in the early morning along with a new cotton layer and leaved it for 24 hours. To avoid the bias by track recognition–between treatments, we interspersed a rest period of 24-hour between treatments, that is, a day without any treatment, when the artificial nest was removed and cleaned with alcohol. Nests were visited thrice, 3, 8, and 24 hours after setting the treatments to evaluate ant presence. During the visit we recorded the following variables: the temperature in the nests (Extech 42500: Mini IR Thermometer, max resolution 1 ºC), the number of ants in the nest, the ant recruitment to the nest, that is, ant trails to access the bird remains, the total number of vertical trails in the trunk at nest-high, and the activity (ants/min) of the vertical trails within 50 cm of the artificial nest (in 100 cm of the trunk perimeter).

4.2. Breeding data and sample collection

During the fall 2012 we installed a total of 75 wooden nest boxes for passerine birds at RBD (entrance diameter 27 mm; inner dimensions: base 15 x 9 cm, height 24 cm). Nest boxes were attached to the trunk or branches of trees at 1.5 m or higher to avoid disturbance by grazing ungulates. In 2013, there were 75 nest boxes (33 invaded, 42 uninvaded) attached to 25 cork oak trees (11 invaded, 14 uninvaded). Trees were separated by at least 50 m from each other and 2 m from the pathway. Each tree had one nest box hanging from the trunk and two from the branches. Due to the low occupation rate (35%), from 2014 onwards the study area was expanded from 25 to 49 trees (25 invaded, 24 uninvaded), incorporating pines and poplar trees (*Populus* sp.). Thus, one third of the nest boxes (22: 12 invaded, 10 uninvaded) were relocated from one of the branches to the trunk of another tree. Besides, from 2014 nest boxes were protected from predators by attaching a PVC pipe to lengthen 10 cm the entrance (diameter 50 mm, thickness 3 mm), which was coated on the inside at the bottom with an anti-slip layer made by a mixture of sand and glue.

Tree selection was limited by the invasion of the Argentine ant and tree trunks were visited before setting the nest boxes to verify its presence. Invaded trees were considered when the invasive species was the only ant species observed in the tree trunk, which is the normal state when the Argentine ant invades this area (Angulo *et al.* 2011; Carpintero *et al.* 2005). To reduce bias derived from the nest box location (e.g. habitat, food resource proximity), invaded and uninvaded trees were interspersed (Fig. 28).

We monitored with routine checks the occupation of nest boxes by great tits and their breeding from 2013 to 2017. We compiled data for each nest box on location (latitude, longitude), laying date, clutch size, brood size, productivity (i.e. number of fledglings), hatching success, fledgling success, and breeding success (see Box 2). Laying and hatching dates were estimated when no directly recorded, by assuming one egg laid per day, 13 complete days of incubation starting when the last egg was laid, synchrony of hatchlings on day 13th, and fledglings abandoned the nest approximately when 17 days old. Only the first broods were considered, and those occupied first by co-occurring bird species were excluded, i.e. *Cyanistes caeruleus* (three in invaded trees, three in uninvaded trees) and *Passer domesticus* (one in an invaded tree, one in an uninvaded tree). Due to National Park restrictions (forbidden access to breeding areas of certain protected bird species), some of the nest boxes could not be monitored as planned. Unsuccessful breeding events caused by circumstances other than the Argentine ant were excluded from the analyses (12 predation events, a human disturbance event, and a nest box found on the ground by unknown causes).

Box 2. Glossary – Breeding variables

Occupied nest: nest with at least one laid egg.
Incubated nest: occupied nest with complete clutches that were warm to the touch on subsequent days.
Hatched nest: incubated nest with at least one hatched egg.
Successful nest: occupied nest with at least one fledgling leaving the nest.
Failed nest: occupied nest that failed by causes different from predation or disturbance events different from the Argentine ant. The sum of all unsuccessful nesting attempts (predated, disturbed, and failed) is equal to the difference between occupied and successful nests.
Laying date: the date the first egg was laid, taking the 1st of March as day 1 in each breeding season (to allow for comparison among years).
Clutch size: number of laid eggs from a complete clutch.
Brood size: number of hatchlings from hatched nests.
Fledgling size (productivity): number of nestlings that fledge and leave successfully the nest (from nest boxes with fledglings).
Occupation success: percentage of occupied nest boxes (excluding those occupied by other species).
Hatching success: percentage of hatchlings to eggs laid (clutch size).
Fledgling success: percentage of fledglings to hatchlings (brood size).
Breeding success: percentage of fledglings to eggs laid (clutch size).
Nest weight: dry weight of the successful nests (subsequent to Berlese).

During the breeding season 2014 nestbox monitoring was intensified during hatching and fledging to record data on chicks and adults. Chicks were banded, measured, and sampled for blood following standard procedures at 10 days of age, so body mass and biometry (wing and tarsus length) could be compared. However, since not all chicks could be sampled for blood at this age, blood sampling was postponed in nests with small siblings, and afterwards days since hatching was incorporated in the models to control for any potential bias. Adults were also captured to band, weight, and take morphometric measurements and blood samples. Blood samples were taken from the jugular vein using a heparinized syringe (< 1% mass) and kept cool (~ 4 °C) until they were centrifuged (4,000 rpm, 20 min, 20 ºC). Blood plasma was separated from the cellular fraction and both were stored at -80 ºC until analyses. Besides, nest weight was obtained as a proxy of female quality (Broggi and Senar 2009). Successful nests were collected, dried at 60 ºC for 7 days, and weighted to the nearest 0.1 g (Acculab ALC-2100).

Blood samples were analyzed to study the nutritional state and oxidative balance of both, chicks and adults. A total of ten parameters, including antioxidant metabolites and enzymes, were obtained from blood plasma and cell package: triglycerides (TGR), cholesterol (CHOL), total proteins (TP), uric acid (UA), total antioxidant capacity (TEAC), thiobarbituric acid reactive substances (TBARS), catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), and glutathione peroxidase (GPX). All **plasma metabolites (TGR, CHOL, TP, and UA)** were measured according to standard methods implemented on a Cobas INTEGRA 400 plus Chemistry autoanalyzer (Roche Diagnostics Ltd. Burgess Hill, West Sussex, UK).

Total antioxidant capacity (TEAC) (mM/l) is a measure of the capacity of plasma to neutralize reactive oxygen species, and was measured following Erel (2004). Recent studies point out that TEAC is mostly representative of the water-soluble components of the antioxidative system (Cohen *et al.* 2009). However, it is commonly agreed that measurement of TEAC in combination with other fat-soluble antioxidants may provide a more complete image of the antioxidant system than TEAC alone (Monaghan *et al.* 2009). **Thiobarbituric acid reactive substances (TBARS)** (nmol MDA /ml) are a by-product of lipid peroxidation and have been measured as described in Buege and Aust (1978). We further determined the activity of four antioxidant enzymes in the cell package. **Catalase (CAT)** (U/mg protein), which catalyzes the decomposition of hydrogen peroxide to water and oxygen and has been measured indirectly through its catalytic activity following Cohen *et al.* (1969). **Superoxide dismutase (SOD)** (U/mg protein) is an antioxidant defense, which catalyzes the dismutation of the superoxide radical into oxygen or hydrogen peroxide and has been measured as described in

McCord and Fridovich (1969). **Glutathione peroxidase (GPX)** (mU/mg protein) is an enzyme family that reduce lipid hydroperoxides to alcohols and hydrogen peroxide to water and has been measured as described in Carmagnol *et al.* (1983). **Glutathione reductase (GR)** (U/mg protein) is an enzyme that catalyzes the reduction of glutathion disulfide (GSSG) to the sufhydryl for glutathione (GSH), which is a critical molecule in resisting oxidative stress and has been measured as described in Cribb *et al.* (1989).

Sample analyses were performed at the Ecophysiology Laboratory at the Doñana Biological Station – CSIC (LEF-EBD; http://ebd.csic.es/lef/web/english/index_e.php) certified to ISO9001:2015 and ISO14001:2015 quality and environmental management systems, respectively.

4.3. Statistical analyses

The Argentine ant attraction to artificial avian nests

We first looked at the normal activity of the ants focusing only on the control treatments, empty nests and nests with untouched eggs. We quantified differences between invaded and uninvaded areas, between oaks and pines, at different times during the survey, and the effect of the temperature and trunk perimeter. We used as dependent variables three estimations of ant activity in the trees (not in the artificial nests): the total number of vertical trails in the trunk relative to the trunk perimeter (trails/m), the ant activity in all vertical trails in 100 cm of the trunk perimeter (ants/min) and the ant activity of the trail having the maximum ant activity (ants/min). We performed three independent generalized linear mixed models for each dependent variable with a Poisson distribution and logit error. The main independent variables were the invasion status (invaded or uninvaded), the type of tree (pine or cork oak), the time of the survey (3, 8 or 24 hours after the set-in), the temperature, and the trunk perimeter. We added the tree as a random factor.

Then, we analyzed differences in attraction to the artificial nest treatments. First, the dependent variable was the relative number of ants in the artificial nest (with respect to the maximum number of ants found in any artificial nest in invaded – 350 ants – and in uninvaded – 190 ants – areas), and it was analyzed with a linear mixed model. The main independent variables were the treatment (five categories), the invasion status (invaded or uninvaded) and the type of tree (pine or cork oak) and second order interactions among these. Differences among categories of the treatment were tested with planned comparisons ("contrast" command, PROC GENMOD, SAS Institute Inc. 2011). Temperature in the nest, trunk perimeter, and the number of ant trails in the trunk relative to the perimeter were included as covariates. We also added tree identity as a random factor. Second, we explored the cases in which there was ant recruitment to any nest remains, analyzing recruitment frequency for each treatment in invaded and uninvaded areas through a generalized linear model with a binomial distribution and logit error. The dependent variable was the number of recruitments seen for each artificial nest from the total survey times (three). We included as independent variables the treatment, the invasion status and the type of tree, and the mean temperature and the trunk perimeter as covariates. Differences between categories of the treatment were tested with planned comparisons ("contrast" command, PROC GENMOD, SAS Institute Inc. 2011).

Breeding performance

Breeding parameters were analyzed with generalized linear mixed models to test the effects of the Argentine ant invasion status (invaded vs. uninvaded) on a set of dependent variables inherent to each nest box (see Box 2 for variables description). Dichotomous variables and proportions were analyzed with binomial distribution: occupation, success, hatching success, fledgling success, and breeding success. Discrete variables were analyzed with Poisson distribution: laying date, clutch size, brood size, and fledgling size (productivity). Nest weight of successful nests was analyzed with a linear model because only data on 2014 was recorded. All models included the invasion status as fixed factor and nest box latitude and longitude as covariates to account for nest box and tree location. Latitude and longitude were rescaled by subtracting the minimum value and multiplied by a thousand to avoid convergence failure in the models. Year was included as random factor in the generalized linear mixed models to account for year-to-year variation in climatic or other uncontrolled factors. Although nest box location was taken into consideration, all analyses were repeated excluding the first year of observations due to nest box relocation (see 4.2).

Individual biometric, biochemical, and oxidative stress parameters

We studied the correlation among individual measurements of mass, biometry, biochemistry, and oxidative stress parameters. In order to summarize biometric and blood parameters into single independent factors we performed a principal component analyses. Although we found correlation among some of the variables (Table 8a), cumulative explained variance by two factors was low (0.48, Table 8b), and did not improve sufficiently by increasing the number of factors (up to four, cumulative proportion < 0.75). Since individual variables were not explained with a multivariate analysis, we analyzed each parameter independently by performing univariate analyses.

We tested the effect of the Argentine ant invasion status (invaded vs. uninvaded) on the following parameters of fledglings: mass, ratio wing/tarsus length, TRG, CHOL, TP, UA, TEAC, TBARS, CAT, SOD, GR, and GPX (see 4.2 for variables description). Since all parameters could not be measured from all blood samples due to variation in volume of extracted blood, sample sizes changed slightly. We ran several independent linear mixed models on each parameter in order to find which factors and covariates better explained the variance observed, including the number of fledglings (NF), the laying date (LD), and the geographical coordinates (latitude and longitude). All models included the invasion status as fixed factor, the age of the chick as covariate to control for different age at the time of sampling, and the nest box as random factor to account for non-independence of data on siblings. Besides, a null model was performed to each measured variable (no variables, only the random factor). Final models were chosen following the Akaike Information Criterion ($\Delta AIC \leq 2$) (Table 9). Response variables were transformed to follow model assumptions (TRG, CHOL, TEAC, TBARS, and GR were log-transformed and wing/tarsus ratio, SOD and GPX were square root-transformed). Only data on fledglings from successful nest boxes was analyzed. Lack of autocorrelation was tested for every model.

Data on adults was analyzed separately by sexes. We tested the invasion status (invaded vs. uninvaded) with an independent linear model for each variable: mass, wing and tarsus length, TRG, CHOL, TP, UA, TEAC, TBARS, CAT, SOD, GR, and GPX. Variables were transformed to fulfil model assumptions. Only data on adults from successful nest boxes was analyzed.

Data on breeding performance and individual parameters were analyzed under R version 3.3.3 (R Core Team 2017). Linear models were analyzed using the function lm, and linear mixed models and generalized linear mixed models with functions lmer and glmer, respectively (package lme4, Bates *et al.* 2015). Variables correlation was calculated using function rcorr (package Hmisc, Harrell and Dupont 2017) and principal component analyses with function rda (package Hmisc, Oksanen *et al.* 2017).

Results and Discussion

Section 1: Native predators living in invaded areas: responses of terrestrial amphibian species to an Argentine ant invasion

Results

Amphibian abundance in invaded and uninvaded areas

Over a total of 342 trap nights, 174 amphibians of 4 different species were caught: 124 natterjack toads (*E. calamita*), 27 western spadefoot toads (*P. cultripes*), 15 stripeless tree frogs (*H. meridionalis*), and 8 Iberian painted frogs (*D. galganoi*) (Fig. 12a). All were adults, except for most of the *E. calamita* captured in the spring (90 juveniles and 5 adults) and most of the *D. galganoi* (6 juveniles) (Table 3a, Fig. 12a).

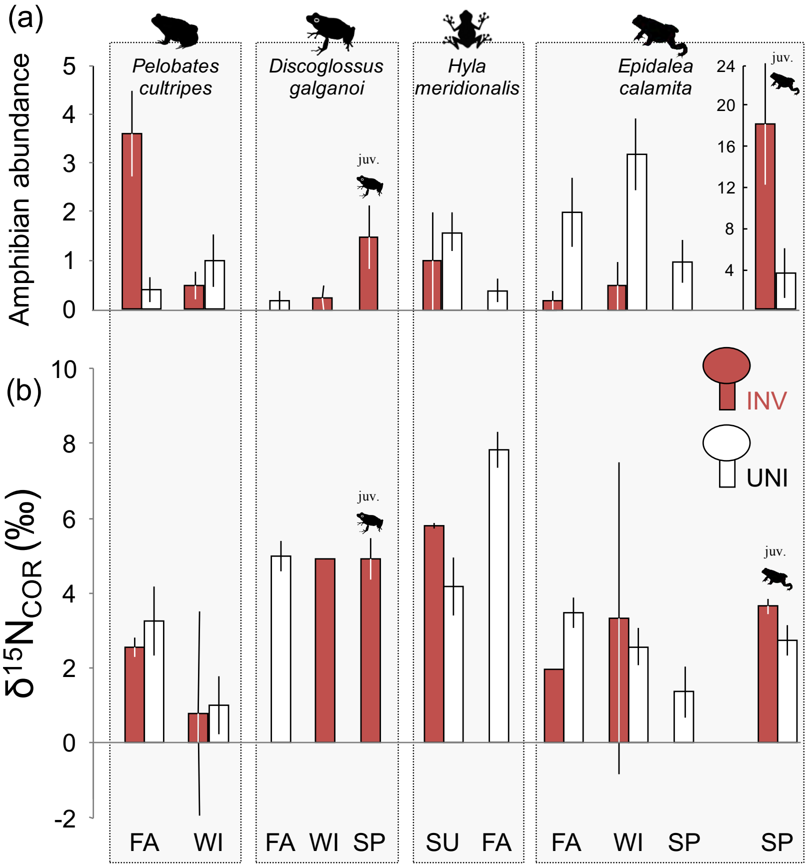


Fig. 12. (a) Number of amphibians captured (mean ± SE) and (b) the $\delta^{15}N_{COR}$ values of amphibian livers (mean ± SE) for invaded and uninvaded areas (INV in red and UNI in white, respectively) across different seasons (SU summer, FA fall, WI winter, SP spring). Data for adults and juveniles are separated (juveniles are specified with "juv."). Only seasons for which abundance was greater than zero in at least one tree are represented.

Table 3. Nitrogen isotopic values of the amphibians captured and the plant species sampled (used as baseline). Nitrogen isotopic values (mean and standard error: $\delta^{15}N$ [SE]) of (a) the amphibian species and (b) the plant species collected in the tree areas in invaded versus uninvaded areas (INV: yes [Y] or no [N], respectively) for each season. The means and standard errors of amphibian total length (mm) and mass (g) are provided; *n* is the sample size for the isotopic analyses, while the numbers of amphibians captured (NTr), stomachs studied (NSt), and empty stomachs (NEm) are also provided. FOR indicates the percentage of non-empty stomachs that contains Formicidae.

Species			**Season**	**Inv**	***n***	**$\delta^{15}N$ (SE)**	
(a)	**Length**	**Mass**					
E. calamita	46.4 (0.8)	11.3 (0.6)	FALL	N	*9*	4.22	(0.44)
				Y	*1*	4.53	-
			WINTER	N	*16*	2.73	(0.54)
				Y	*2*	4.66	(4.16)
			SPRING	N	*5*	2.98	(0.89)
E. calamita (juv)	16.3 (0.4)	0.6 (0.1)	SPRING	N	*6*	5.18	(0.35)
				Y	*24*	5.94	(0.17)
P. cultripes	39.1 (1.7)	9.2 (1.5)	FALL	Y	*16*	5.26	(0.18)
				N	*2*	4.46	(0.03)
			WINTER	Y	*2*	2.69	(3.31)
				N	*5*	0.99	(0.77)
D. galgonoi	39.3	8.1	FALL	N	*1*	6.53	-
	42.5	9.6	WINTER	Y	*1*	7.40	-
D. galgonoi (juv)	20.2 (0.4)	0.6 (0.1)	SPRING	Y	*6*	7.21	(0.48)
H. meridionalis	22.4 (0.7)	0.9 (0.1)	SUMMER	Y	*3*	7.82	(0.08)
				N	*5*	6.57	(0.62)
			FALL	N	*2*	8.23	(0.59)
(b)							
Plants found under *Q. suber* canopy			SUMMER	Y	*44*	1.39	(0.19)
				N	*46*	2.41	(0.28)
			FALL	Y	*44*	2.70	(0.22)
				N	*52*	1.05	(0.28)
			WINTER	Y	*37*	1.25	(0.26)
				N	*50*	0.30	(0.29)
			SPRING	Y	*38*	2.03	(0.27)
				N	*51*	1.63	(0.23)

In the case of *D. galganoi* and *H. meridionalis*, the numbers of adults captured did not differ based on invasion status or season, nor was the interaction between variables significant (*D. galganoi* χ_1^2 = 0.03, p = 0.860; χ_3^2 = 2.05, p = 0.561; and χ_3^2 =2.05, p = 0.561, respectively; *H. meridionalis* χ_1^2 = 1.05, p = 0.306; χ_2^2 = 5.05, p = 0.080; and χ_3^2 = 3.11, p = 0.374, respectively). *D. galganoi* and *E. calamita* juveniles occurred in higher numbers in invaded areas, but neither season nor the invasion-by-season interaction was significant (*D. galganoi* χ_1^2 = 4.11, p = 0.043; χ_2^2 = 4.19, p = 0.123; χ_2^2 = 4.19, p = 0.123, respectively; *E. calamita* χ_1^2 = 3.92, p = 0.048; χ_2^2 = 5.99, p = 0.050; χ_2^2 = 3.99, p = 0.136, respectively). In the case of *P. cultripes* adults, invasion status and season were marginally significant, but their interaction was not significant (χ_1^2 = 3.70, p = 0.054; χ_3^2 = 7.70, p = 0.053; and χ_3^2 = 6.83, p = 0.078, respectively). *P. cultripes* was more abundant in invaded areas than in uninvaded areas and in the fall than in the winter (Fig. 12a). In the case of *E. calamita* adults, invasion status was significant, while season and the invasion-by-season interaction were not significant (χ_1^2 = 5.67, p = 0.017; χ_3^2 = 6.99, p = 0.072; and χ_3^2 = 5.92, p = 0.116, respectively). Fewer adults of *E. calamita* were observed in invaded areas (Fig. 12a).

Prey availability

A total of 5,319 non-ant invertebrates and 22,386 ants (mostly Argentine ants) were captured in invaded areas. In uninvaded areas, 6,545 non-ant invertebrates and 4,614 native ants were captured; no Argentine ants were present. Beetles and millipedes accounted for more than 40% of the available biomass across all seasons, except in the winter, when flies were more abundant than millipedes (Fig. 13a). Total available biomass was nearly significantly different across seasons but was not affected by invasion status or the invasion- by-season interaction (χ_3^2 = 7.48, p = 0.058; χ_1^2 = 3.32, p = 0.068; χ_3^2 = 6.26, p = 0.100, respectively; N = 114).

A total of 27,000 ants were captured, of which 22,381 were Argentine ants (Table 4a). In uninvaded areas, 14 ant species were found in and under trees (Fig. 13b). Only Argentine ants were found in invaded areas (except for *Temnothorax* sp., which appeared in the summer in two invaded areas), and only native ants were found in uninvaded areas. The percentage of available ant biomass differed significantly between invaded and uninvaded areas and across seasons, but the interaction between the two factors was not significant (χ_1^2 = 6.35, p = 0.012; χ_3^2 = 8.34, p = 0.040; and χ_3^2 = 6.62, p = 0.085, respectively; N = 114). Ant biomass was greater in invaded versus uninvaded areas (Table 4a).

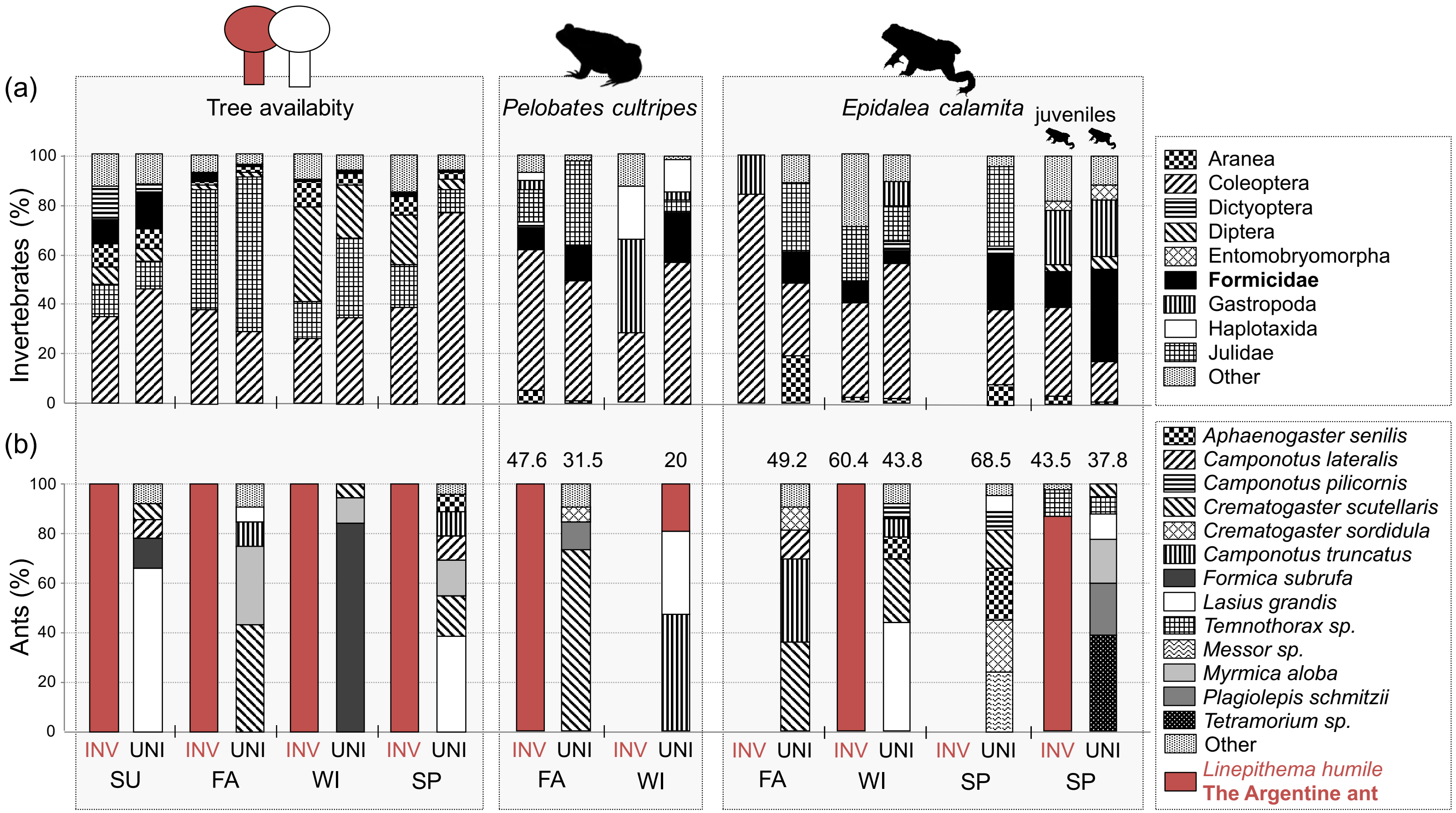


Fig. 13. Mean percentage of (a) invertebrate biomass and (b) ant species biomass (other = ant species < 5 % of relative ant biomass). The figures represent relative availability as estimated from pitfall traps ( ) and relative presence in amphibian stomach contents for the different seasons in invaded (INV) versus uninvaded (UNI) areas. In (b), the numbers above the bars indicate the percentage of ant items out of all the invertebrates found in amphibian stomachs.

Table 4. Ant species environmental availability and presence in stomach contents (biomass and abundance) Percentage (mean ± SE) of ant species biomass (a) available in the environment versus (b) in amphibian stomach contents across the four seasons in invaded and uninvaded areas (INV column, Y or N respectively). N is the number of stomachs that contained ants. BiomassTOT is the total biomass available in the environment (in a) or consumed (in b), and BiomassANT is the total ant biomass (g, mean ± SE). Ntot is the total number of ants, and BIOid is the mean biomass of one ant (g). Ant species abbreviations are as follows: *Linepithema humile* (*L. hum*), *Aphaenogaster senilis* (*A. sen*), *Camponotus aethiops* (*C. aet*), *Camponotus lateralis* (*C. lat*), *Camponotus piceus* (C.pic), *Camponotus pilicornis* (*C. pil*), *Cataglyphis tartessica* (*C. tar*), *Crematogaster scutellaris* (*C. scu*), *Crematogaster sordidula* (*C. sor*), *Camponotus truncatus* (*C. tru*), *Formica subrufa* (*F. sub*), *Hypoponera eduardi* (*H. edu*), *Lasius grandis* (*L. gra*), *Temnothorax sp.* (*Tem. sp*), *Messor sp.* (*M. sp*), *Myrmica aloba* (*M. alo*), *Plagiolepis schmitzii* (*P. sch*), and *Tetramorium sp.* (*Tet. sp*).

SPECIES	SEASON	INV	*N*	BiomassTOT	BiomassANT	*L.hum*	*A.sen*	*C.aet*	*C.tru*	*C.lat*	*C.pic*	*C.tar*	*C.scu*	*F.sub*	*H.edu*	*L.gra*	*Tem.sp.*	*M.alo*	*P.sch*	*Tet.sp.*	*C.pil*	*M.sp.*	*C.sor*
					BIO id	0.08	1.35	0.57	0.4	0.59	0.67	0.55	0.59	1.24	0.15	0.28	0.1	0.7	0,06	0,06	0,67	0,05	0,59
(a)																							
Q. suber	SUMMER	Y	5	195.5 (58.7)	66.4 (19.4)	100	-	-	-	-	-	-	-	-	-	-	<0.1	-	-	-	-	-	-
Q. suber	SUMMER	N	5	285.8 (72.8)	99 (24.1)	-	<0.1	0.2 (0.1)	1.4 (0.3)	6.5 (1.5)	-	-	6.4 (3.1)	7.2 (5.3)	-	64.8 (24.1)	0.2 (0.1)	12.2 (6.4)	<0.1	<0.1	-	-	-
Q. suber	FALL	Y	5	115.8 (45.4)	44.7 (16.4)	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Q. suber	FALL	N	5	11.6 (2.8)	5.4 (1.3)	-	1.8 (1.7)	-	9.4 (2.6)	4.2 (3.1)	4.2 (2.6)	-	40.8 (19.4)	3.2 (3.1)	0.5 (0.4)	5.8 (1.3)	0.3 (0.2)	29.5 (20.6)	<0.1	0.3 (0.1)	-	-	-
Q. suber	WINTER	Y	4	2.3 (0.4)	0.9 (0.1)	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Q. suber	WINTER	N	5	4.1 (1.1)	1.4 (0.4)	-	-	-	-	-	-	-	5.8 (3.4)	82.8 (25.4)	-	-	-	10.3 (6.7)	-	-	-	-	-
Q. suber	SPRING	Y	4	7.3 (2.1)	3 (0.8)	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Q. suber	SPRING	N	5	10.5 (3.2)	4 (1.1)	-	6.8 (6.8)	-	9.3 (4)	9.9 (3.3)	-	1.6 (1)	15.7 (6.9)	4.1 (4.1)	-	37.3 (11.4)	-	14.1 (11.4)	<0.1	1 (0.5)	-	-	-
					Ntot	22,381	5	4	84	198	5	2	237	105	2	3,614	33	310	6	14	-	-	-
(b)																							
E. calamita	FALL	N	8	98 (37.9)	9.3 (3)	-	-	-	33 (16)	11 (11)	-	-	36 (17)	-	-	<1	2 (2)	-	-	1 (1)	-	5 (3)	10 (10)
E. calamita	WINTER	N	14	159.1 (28.7)	5.1 (1.6)	-	9 (6)	-	8 (7)	-	-	-	26 (11)	-	-	44 (10)	1 (1)	-	-	1 (1)	6 (4)	5 (3)	1 (1)
E. calamita	WINTER	Y	2	37.2 (22.4)	0.6 (0.52)	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. calamita	SPRING	N	5	142.4 (24.4)	30.7 (17.4)	-	21 (9)	-	3 (3)	2 (2)	-	-	15 (10)	-	-	7 (4)	1 (1)	-	-	-	7 (7)	24 (18)	21 (13)
E. calamita (juv)	SPRING	N	5	10 (2.7)	2.6 (0.5)	-	-	-	-	-	-	-	7 (7)	-	-	10 (10)	7 (7)	18 (18)	21 (20)	38 (20)	-	-	-
E. calamita (juv)	SPRING	Y	24	11.6 (1.4)	1.2 (0.2)	88 (5)	-	-	-	-	-	-	-	-	-	-	11 (5)	-	1 (1)	1 (1)	-	-	-
P. cultripes	FALL	Y	1	182.5	1.35	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. cultripes	FALL	N	1	69.2	30.9	-	-	-	-	-	-	-	73	-	-	4	1	4	12	-	-	-	6
P. cultripes	WINTER	N	1	0.84	0.83	19	-	-	48	-	-	-	-	-	-	33	-	-	-	-	-	-	-
D. galgonoi	FALL	N	1	47.23	31.88	-	-	-	46	-	-	-	44	-	-	3	1	2	-	-	-	-	4
H. meridionalis	SUMMER	N	2	5.3 (0.6)	1.2 (0.4)	-	-	-	-	-	-	-	-	-	-	100	-	-	-	-	-	-	-
H. meridionalis	SUMMER	Y	2	6 (0.9)	0.6 (0.5)	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H. meridionalis	FALL	N	2	24.3 (4.7)	5.2 (2.1)	-	-	-	5	-	-	-	86	-	-	9	-	-	-	-	-	-	-
					Ntot	360	19	0	62	23	0	0	146	0	0	183	41	4	41	29	14	246	38

Amphibian diet in invaded and uninvaded areas

Stomach contents were obtained from 112 amphibians (9.8% had empty stomachs, Table 3a). However, isotopic samples were obtained from 106 individuals because the liver samples from six individuals had deteriorated. The cumulative prey curve for the two major amphibian species, *E. calamita* (adults and juveniles) and *P. cultripes*, reached a well-defined asymptote, indicating that the sample size was sufficient to adequately describe the amphibians' diets (Fig. 14). For *H. meridionalis* and *D. galganoi*, sample sizes were lower, but the results are nonetheless provided for the sake of comparison.

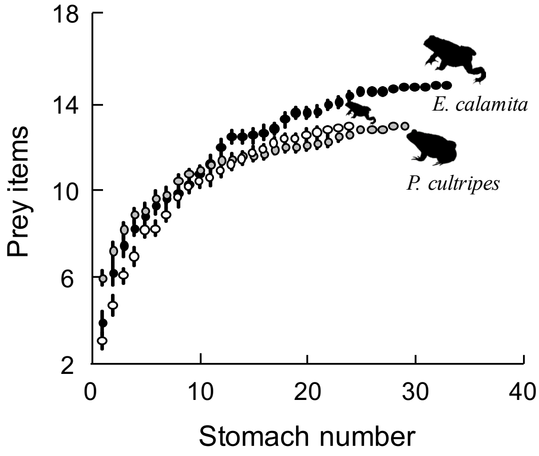


Fig. 14. Cumulative prey curve. Randomized cumulative prey curves for *E. calamita* (adults and juveniles) and *P. cultripes*. Mean values for 10 randomized trials are presented (± SE).

Based on their stomach contents, the amphibians had varied diets; they consumed nine different taxonomic groups (Fig. 13a). In terms of total biomass, Coleoptera was by far the amphibians' most frequent prey (Fig. 13a); they made up 16-84% of their diets (except in the case of *D. galganoi*). Indeed, Formicidae was the second or third most frequently consumed group (up to 37% of dietary biomass). Total consumed biomass did not differ based on invasion status, species, or season ($\chi_1^2 = 0.01$, p = 0.933; $\chi_3^2 = 2.09$, p = 0.555; and $\chi_3^2 = 5.23$, p = 0.156, respectively; N = 95).

Ants were found in almost all the stomachs of *E. calamita* adults and juveniles and *H. meridionalis adults* (32/34, 30/30, and 9/12, respectively; Table 3a). Formicidae was less common in *P. cultripes* stomachs (both in terms of biomass and frequency). Ant frequency, mean percentage of consumed ant biomass, and the number of ant species consumed were greater for *E. calamita* (adults and juveniles) than for other species (Fig. 13a, Table 4b). Thus, of the amphibians studied, *E. calamita* showed the greatest degree of ant specialization. Thirteen species of Formicidae were observed in the stomach contents: 12 native species and the Argentine ant (Table 4b). Except in one individual, Argentine ants were the only ant species found in adult amphibians from invaded areas. Conversely, except in one individual, Argentine ants were completely absent from the stomachs of amphibians from uninvaded areas (Fig. 13b). Invasion status did have a significant effect on the percentage of ant biomass consumed ($\chi_1^2 = 5.04$, $p = 0.025$, $N = 95$). Significantly more ant biomass was consumed in uninvaded areas than in invaded areas (8.15 ± 2.3 versus 0.86 ± 0.2 g, respectively). Season and species did not have an effect ($\chi_3^2 = 1.87$, $p = 0.600$, and $\chi_3^2 = 1.98$, $p = 0.577$, respectively; $N = 95$).

The nitrogen isotopic baseline was significantly different across seasons and individual tree areas ($\chi_3^2 = 25.43$, $p < 0.001$ and $\chi_9^2 = 18.11$, $p = 0.034$, respectively; $N = 362$, Table 3b). This finding meant that the amphibians' nitrogen isotopic values needed to be corrected. Amphibian nitrogen isotopic ratios did not differ between invaded and uninvaded areas, among species, or across seasons (invasion status $\chi_1^2 = 3.10$, $p = 0.078$; amphibian species $\chi_5^2 = 7.52$, $p = 0.185$; season $\chi_3^2 = 4.44$, $p = 0.218$; $N = 106$); the interaction between invasion status and species was not significant ($\chi_4^2 = 3.05$, $p = 0.549$, $N = 106$) (Fig. 12b). The values were highly variable, which probably explains why no effect of invasion status was found.

Preference tests

Similar results were obtained from the two types of preference tests (providing the adult toad with four ant species simultaneously or each ant species separately). *E. calamita* adults ate both native ants and Argentine ants. However, they ate native ants (*Crematogaster scutellaris*, *Aphaenogaster senilis* and *Tapinoma* cf. *nigerrimum*) faster and in greater quantities (Fig. 15a). When the amphibians were simultaneously offered the four ant species, there were ant-species-specific differences in consumption ($\chi_3^2 = 406.34$, $p < 0.0001$, $N = 1{,}400$). Fewer Argentine ants were eaten: at 30 min, around 50% of Argentine ants were left versus fewer than 30% of native ants (Fig. 15a). When we compared survivorship, the Argentine ant survived longer than the native ants: 2.03 times longer than *C. scutellaris*, 5.17 times longer than *A. senilis*, and 5.42 times longer than *T.* cf. *nigerrimum*. When the amphibians were offered one ant species at a time, there were again ant-species-specific differences in consumption ($\chi_3^2 = 146.72$, $p < 0.0001$, N = 1,120): 30% of Argentine ants remained at 30 min versus less than 20% of native ants. Once again, the Argentine ant survived longer than the native ants: 2.31 times longer than *C. scutellaris*, 2.59 times longer than *T.* cf. *nigerrimum*, and 2.78 times longer than *A. senilis*. Furthermore, in the second test, no ants were eaten in six of the trials; the percentage of trials in which no ants were eaten was 21.4% for the Argentine ant (3 trials), 14.3% for *T.* cf. *nigerrimum* (2 trials), 7.1% for *C. scutellaris* (1 trial), and 0% for *A. senilis*.

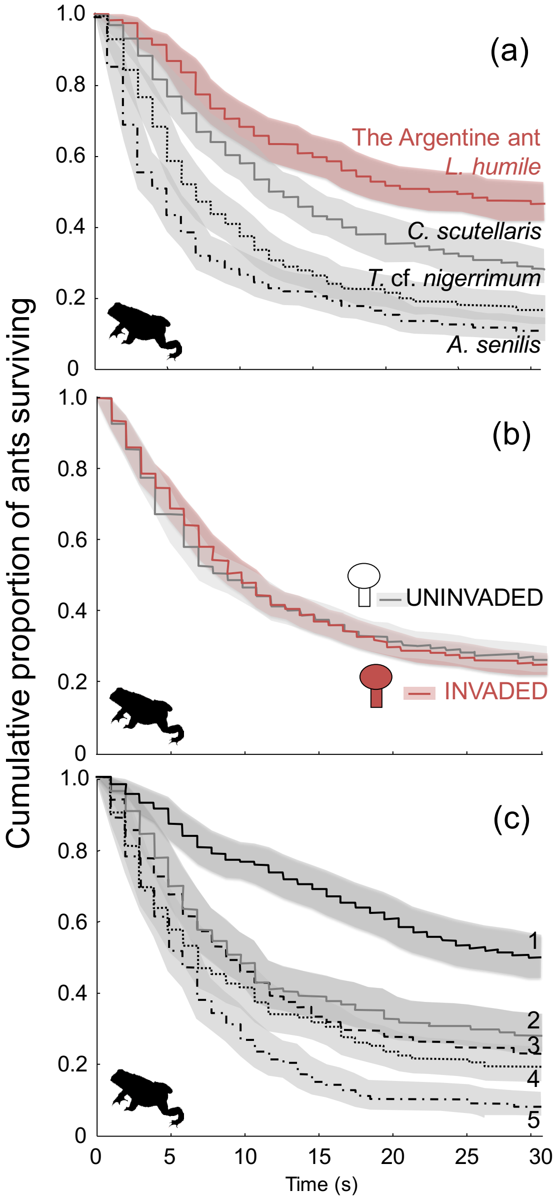


Fig. 15. Ant preferences demonstrated by *Epidalea calamita*. Consumption of live ants over the course of the first trial (simultaneous exposure to four ant species): (a) by each ant species; (b) for Argentine ants exposed to *E. calamita* adults from invaded versus uninvaded areas; and (c) by trial order (1st to 5th; all ants). Shaded areas represent 95% CI.

Amphibians from invaded versus uninvaded areas did not differ in their rates of Argentine ant consumption ($\chi_1^2 = 6e{-}04$, $p = 0.981$, N = 1,400 observations, Fig. 15b). Both trial order and the ant-species-by-trial-order interaction were significant, meaning that there was a learning process and a significant difference among ant species in the relative degree of learning ($\chi_1^2 = 37.81$, $p < 0.0001$; $\chi_3^2 = 12.831$, $p = 0.005$, respectively, N = 1,400 observations). Toads fed on each ant species faster in subsequent trials (*L. humile* $\chi_1^2 = 22.94$, $p < 0.001$, Fig. 15c; *A. senilis* $\chi_1^2 = 34.06$, $p < 0.001$; *T.* cf. *nigerrimum* $\chi_1^2 = 20.10$, $p < 0.001$; *C. scutellaris* $\chi_1^2 = 28.82$, $p < 0.001$; N = 350).

Discussion

Although none of the terrestrial amphibians at RBD exclusively consume ants, ants do constitute a significant percentage of their diets. Indeed, when we considered the relative representation of ants in amphibian diets, as compared to other invertebrate taxa, we found that amphibians in invaded areas consumed less ant biomass than amphibians in uninvaded areas, even though more ant biomass was available in invaded areas (but comprised only Argentine ants). These differences in consumption could be the result of a preference for native ants over Argentine ants (even post exposure). Because levels of available and consumed invertebrate biomass were similar between invaded and uninvaded areas and amphibians in invaded areas consumed less ant biomass, amphibians shifted to non-ant prey in invaded areas. The Argentine ant invasion also seems to have differentially affected the abundances of adult amphibians. While *H. meridionalis* and *D. galganoi* appeared to be unaffected, *P. cultripes* was more common in invaded areas, although this difference was less pronounced in the winter than in the fall. In contrast, *E. calamita*, the greatest ant specialist in the amphibian community, seemed to avoid invaded areas.

Effects on the amphibian community

The Argentine ant is already established in some suitable habitats in Doñana, where it has replaced most native ant species by competition (Angulo *et al.* 2011; Carpintero *et al.* 2007; Carpintero *et al.* 2005). According to the best known hypotheses that examine the potential relationships between invasive prey and native predators

(Callaway and Ridenour 2004; Carlsson *et al.* 2009; Catford *et al.* 2009; Ricciardi *et al.* 2013; Sax *et al.* 2007), native predators are more likely to be negatively impacted if they display greater dietary specialization. Our stable isotopic analyses confirm that Doñana's terrestrial amphibian community displayed less dietary specialization than expected: δ^{15}N liver tissue values were highly variable even though the nitrogen isotopic baseline was relatively stable (Post 2002; Vanderklift and Ponsard 2003). However, ants may nonetheless make up a significant percentage of their diets (the percentage of consumed ant biomass was much greater than the percentage of available ant biomass).

To date, Argentine ant invasions had only been found to reduce predator abundance in the case of the coastal horned lizard, *Phrynosoma coronatum*, a highly specialized predator of ants (Fisher *et al.* 2002; Suarez *et al.* 2000). In contrast, other ant specialists seem to have benefitted from the abundant food that stems from Argentine ant invasions (Glenn and Holway 2008; Touyama *et al.* 2008). In this study, we found differential effects of the invasion on adult amphibian abundance, which could be related to the species' degree of dietary specialization. The amphibian that consumed the smallest percentage of ants, *P. cultripes*, has the highest abundance of adults in invaded areas. However, there were no differences in adult abundance between invaded and uninvaded areas for *H. meridionalis* and *D. galganoi*, species that consumed intermediate percentages of ants. In contrast, we discovered that *E. calamita* adults were less abundant in invaded areas. This finding makes sense, given that *E. calamita* (as well as other bufonids; Isacch and Barg 2002) is the greatest ant specialist of the four amphibian species studied. Furthermore, in our study, the number of ants consumed, the mean percentage of ant biomass consumed, and the number of ant species consumed were greatest for *E. calamita*. However, *E. calamita* juveniles occurred in higher numbers in invaded areas than in uninvaded areas. Similar results were seen for *D. galganoi* juveniles. This contrast between adults and juveniles could be explained by the fact that juveniles are unable to choose the ponds from which they emerge, while adults can choose where they forage and breed. The greater abundance of *P. cultripes* adults in invaded areas was counterintuitive. It might be that they were attracted by the greater ant biomass in those areas or by a lower degree of interspecific competition, as other amphibian species seemed to avoid these areas (i.e., *E. calamita* adults). Although these results should be interpreted with caution because of our low amphibian sample sizes and given the difficulties associated with estimating amphibian abundance (i.e., terrestrial amphibians strongly respond to fluctuations in precipitation), we propose that Argentine ant invasions may have an effect on the most ant-specialized amphibian species.

Dietary shifts in the presence of the Argentine ant

Predators may not consume invasive prey if they are naïve, if they are absolute specialists (as per Catford *et al.* 2009), or if invasive prey release toxins. As a consequence, dietary shifts and reductions in prey availability occur, which are some of the mechanisms that explain the negative effects invasive prey species have on predators (Caut *et al.* 2008; Suarez and Case 2002; Suarez *et al.* 2000). In less extreme scenarios, predators should consume large quantities of the invasive prey, at least according to the exotic prey naïveté or increased susceptibility hypotheses; such may also be the case if the predator is an absolute generalist (Catford *et al.* 2009; Colautti *et al.* 2004; Li *et al.* 2011; Wanger *et al.* 2011). The amphibian community we studied here seems to provide an example of a less extreme scenario, as Argentine ants were consumed to some degree. However, the diets of Doñana amphibians clearly reflect the previously described (Angulo *et al.* 2011) negative effects of the Argentine ant on native ant communities: amphibians from uninvaded areas consumed a greater diversity of native ants, which largely corresponded to species availability. In contrast, the Argentine ant was almost the only ant species found in the stomach contents of amphibians from invaded areas, which also corresponded to species availability. Even if certain amphibians, such as *E. calamita* adults, seemed to avoid invaded areas, individuals may remain in them long enough to consume an entire meal of Argentine ants. Adult amphibians can have large foraging areas (Miaud *et al.* 2000), but we only observed two cases in which individuals ate ants that did not correspond to the area in which they were captured.

Previous work at our study site has suggested that myrmecophagous amphibians may include the Argentine ant in their diets (Díaz-Paniagua *et al.* 2005). Indeed, the Argentine ant appears to be consumed by most ant predators, including amphibians (Ito *et al.* 2009), jumping spiders (Touyama *et al.* 2008), and pit-building antlions (Glenn and Holway 2008). In nature, the coastal horned lizard does not consume the Argentine ant and compensates for the elimination of its main prey species (native ants) by consuming greater quantities of other invertebrates (Suarez and Case 2002; Suarez *et al.*

2000). In our study, we found that amphibians consumed significantly smaller percentages of ant biomass in invaded areas than in uninvaded areas. Clearly, amphibians do not completely replace native ants by Argentine ants, even when levels of Argentine ant biomass are higher than those of native ants. Doñana amphibians compensated for the lack of native ants by shifting their diet to include other invertebrates: the total biomass consumed was similar in invaded and uninvaded areas. Because the percentage of ants consumed was lower in invaded areas, the percentage of other invertebrates consumed should be higher.

Amphibian prey preferences

When native predators are faced with novel prey, they may fail to recognize or capture the introduced prey species or may be unable to consume it because it is unpalatable or contains toxins. We found that *E. calamita* adults recognized Argentine ants as prey, capturing and consuming them, albeit at markedly lower rates than for native ants. This result could stem from lower detection probabilities or lesser palatability resulting from the Argentine ant's small size or color, as seen in the case of the coastal horned lizard (Suarez *et al.* 2000). Of the ants tested in the laboratory, the Argentine ant was the smallest, followed by *T.* cf. *nigerrimum* and *C. scutellaris*; *A. senilis* was the largest. *A. senilis* and *T.* cf. *nigerrimum* are black, *C. scutellaris* is two-toned (black and red), and the Argentine ant is sand colored. Thus, although the Argentine ant is most similar to *T.* cf. *nigerrimum*, their survivorship patterns in the preference tests differed dramatically. The Argentine ant's marked dissimilarity in size and color might explain its higher survival rates in the laboratory experiment. In the field, of the 12 native ant species consumed by Doñana amphibians, only one (*Plagiolepis schmitzii*) is smaller than the Argentine ant (Arnan *et al.* 2017). Although prey movement is required to trigger feeding responses in some anurans (Oliver 1955), Doñana amphibians consumed native ants that moved faster (*A. senilis*) and slower (*C. scutellaris* and *Temnothorax* sp.) than the Argentine ant, which suggests that movement does not play a significant role to explain our results. The preference for native ants could be explained by the Argentine ant having a lower energetic value. However, Pekár and Mayntz (2014) recently showed that differences in the nutritional composition of European ants cannot fully explain the preferences of predators. Finally, even if the Argentine ant has antipredatory defenses, such as aggressive behavior or noxious chemicals (Glenn and Holway 2008; Robbins *et al.* 2013; Suarez and Case 2002), they did not alter the response of *E. calamita* toads, which increased their feeding response with greater exposure. It could be that learning is occurring. Robbins *et al.* (2013) also showed that fence lizards learned to eat invasive ants over successive feeding trials. However, in our study, adults of *E. calamita* from invaded and uninvaded areas consumed Argentine ants at similar rates, indicating that prior exposure neither positively nor negatively influenced consumption.

In conclusion, when it comes to interactions between native predators and invasive prey, it is essential to consider both the direct and indirect effects of invaders on the native predator community, which means examining predator diets, prey availability, and predator feeding capacities (e.g., prey preferences, ability to learn). Although many generalist predators include ants in their diets, detailed studies on how predators are affected by Argentine ant invasions are very limited. Research on such bottom–up effects is important if we are to understand the impact of ecologically important invaders at higher trophic levels.

Section 2: Are amphibians threatened by a global invasive ant?

Results

Linepithema humile *and juvenile amphibians overlap at the local scale*

When newly metamorphosed *E. calamita* toadlets were emerging from the temporary ponds, we found that different species of native ants overlapped with toadlets from the uninvaded ponds during the day (Figure 16a). Toadlets coming from the invaded ponds only overlapped with *L. humile*, which was the sole ant species present and was much more abundant than native ants were in uninvaded ponds. Moreover, *L. humile* was also present during the day and part of the night (Figure 16a,b).

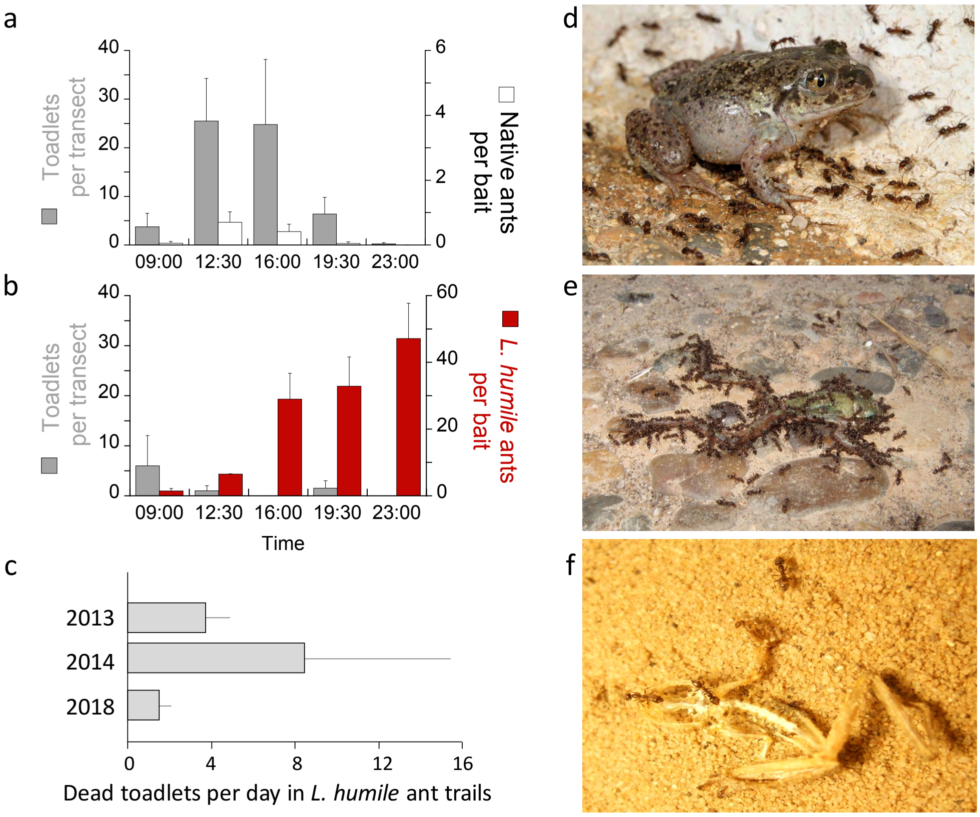


Fig. 16. *Linepithema humile* ants overlap temporally and spatially with amphibians. Relative abundances over time of *Epidalea calamita* toadlets emerging from temporary ponds and (a) native ants or (b) *L. humile* ants. Values represent the mean number (± SE) of toadlets per transect or ants per bait. Note the differences in axis scale between (a) and (b) regarding ants. (c) Mean (± SE) number of dead amphibians found along *L. humile* trails during the juvenile amphibian emergence period over three different seasons. (d-f) Examples of different phases of ant predation on amphibians: (d) ants attack *Pelobates cultripes* toadlet; (e) freshly killed *Hyla meridionalis* covered by *L. humile*, around two hours after an attack; (f) skeleton of an *H. meridionalis* froglet, fewer than 12 h after an attack (*Photo credits: Fernando Amor (d) and Elena Angulo (e,f)*).

Linepithema humile *preys on and kills juvenile amphibians*

We observed dead juvenile amphibians along relatively permanent *L. humile* trails during three different breeding seasons, when newly metamorphosed amphibians of different species were emerging (Figure 16c,d,e,f). All the dead toadlets were found less than 10 cm from the trails (i.e., no other dead toadlets were discovered further away). During the 4 sampling days per season, along 40 m of ant trails, we observed a total of 46 dead *H. meridionalis* frogs (12 in 2013, 34 in 2014); 6 dead *P. cultripes* toadlets (3 in 2013, 3 in 2018); 2 dead Iberian painted frogs (*Discoglossus galganoi*; 2018); and 1 dead Iberian parsley frog (*Pelodytes ibericus*; 2018). We also observed a healthy *P. cultripes* toadlet that began to cross an Argentine ant trail but that could not continue once it came in contact with the ants. The ants were preying on the amphibians, which ranged from having recently died to being entirely eaten (skeletons) (Figure 16e,f).

Linepithema humile *aggressiveness in the ant-trail-exposure experiment*

In the field, *P. cultripes* and *H. meridionalis* juveniles were held next to ant trails for two minutes to allow physical contact with the ants. The juveniles were then set free, and we observed whether they escaped from the trails or remained on them; we also determined their fate during the subsequent 48-h period. None of the observed frequencies (alive, paralyzed, and dead) differed significantly from the expected frequencies ($p > 0.05$ in all cases, Fig. 17a). However, only 60% of the *P. cultripes* toadlets escaped *L. humile* (by jumping away from the trails); 20% died, and another 20% were paralyzed but recovered after approximately 10 min ($N = 10$; Fig. 17a). *H.meridionalis* froglets always escaped ($N = 10$; Fig. 17a). None of the juveniles exposed to the two native ant species (*A. senilis* and *T* cf. *nigerrimum*) showed signs of paralysis; they were all alive at the end of the experiment (Fig. 17a).

Linepithema humile *aggressiveness in the foraging-arena-exposure experiment*

In the laboratory, juveniles of *P. cultripes*, *H. meridionalis*, and *E. calamita* were left in the foraging arenas of colonies of each ant species for 10 min. In these tests, the native ant *A. senilis* was fastest to discover the toadlets, while the invasive ant *L.humile* was the slowest ($\chi^2 = 27.0$, $p < 0.001$, $N = 290$; $p < 0.001$ for all contrasts with

A. senilis). Moreover, juveniles were covered by significantly more ants when exposed to the native ant *T.* cf. *nigerrimum* than when exposed to the invasive ant *L. humile* (mean ± SE: 17.9 ± 1.9 ants vs. 13.0 ± 2.0 ants, respectively; $\chi^2 = 177.22$, $p < 0.001$, N = 284; $p < 0.018$ for all contrasts with *T.* cf. *nigerrimum*; Box 3).

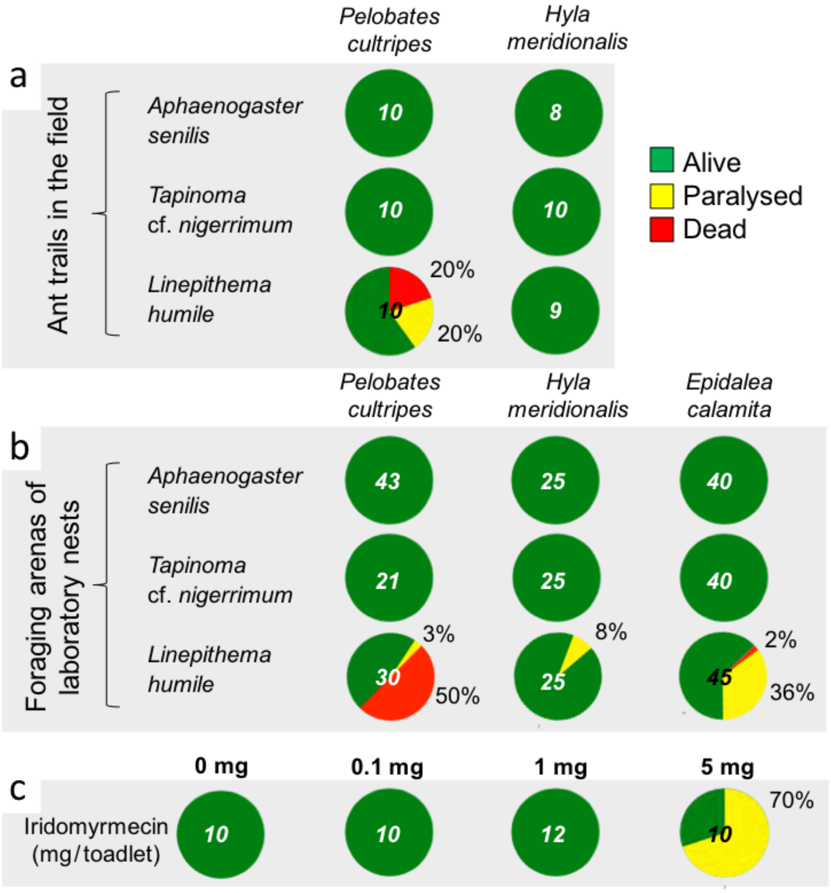
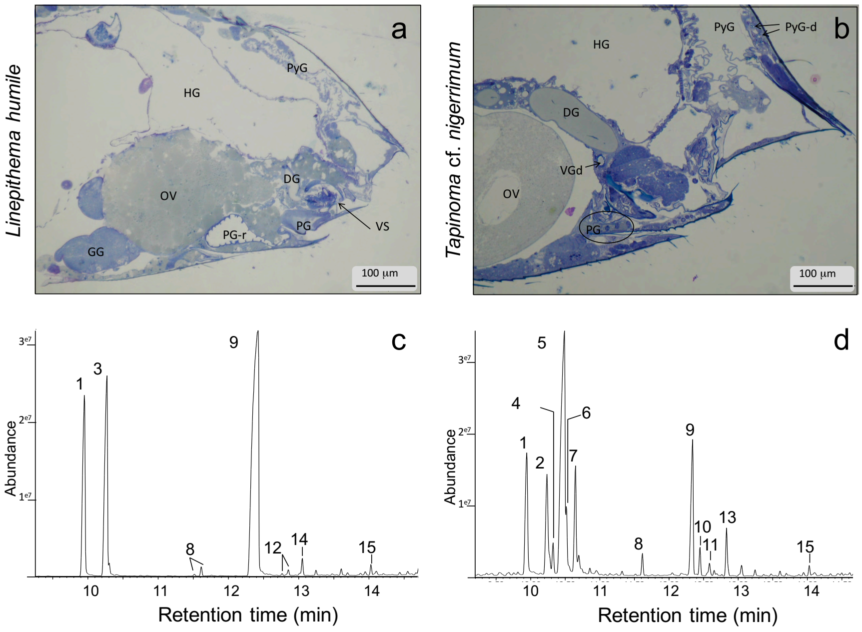


Fig. 17. Effects of ant contact and ant chemicals on juvenile amphibians. (a) Effects on juveniles of two amphibian species that spent 2–10 min in contact with ants on ant trails in the field; (b) Effects on juveniles of three amphibian species that spent 10 min in contact with ants in the foraging arenas of laboratory nests; (c) Effects on *Pelobates cultripes* toadlets whose backs were rubbed with three different concentrations of iridomyrmecin (0.1, 1, or 5 mg/toadlet, which was equivalent to 8.15 ± 1.13, 67.86 ± 6.78, or 307.62 ± 30.30 *Linepithema humile* workers/g of toadlet; mean ± SE). Sample size is indicated in the center of each pie chart.

However, while attacks by the native ants *A. senilis* and *T.* cf. *nigerrimum* had no obvious effect, those by the invader *L. humile* ultimately resulted in paralysis and death. For *P. cultripes*, 53% of toadlets were paralyzed during exposure to the invasive ant *L. humile*, and all but one of these toadlets died within 48 h of the trials (N = 30; Fig. 17b). For *E. calamita*, 38% of toadlets were paralyzed during exposure to the invasive ant *L. humile*, but they recovered ~10 min later; only one died (N = 45; Fig.17b). Finally, for *H. meridionalis*, only 8% of juveniles were paralyzed, and they recovered within 10 min (N = 25). None of the juveniles exposed to the native ants *A. senilis* and *T.* cf. *nigerrimum* showed signs of paralysis; they were all alive at the end of the experiment (Fig. 17b). Indeed, none of the observed frequencies (alive, paralyzed, and dead) differed significantly from the expected frequencies (all individuals alive) for any of the native ant-amphibian species pairs ($p > 0.05$ in all cases, Fig. 17b); this was also true for the *L. humile*—*H. meridionalis* combination. However, there were significant differences for *L. humile*—*P. cultripes* ($\chi^2 = 8.53$, $p = 0.014$) and *L. humile*—*E. calamita* ($\chi^2 = 6.42$, $p = 0.040$).

Iridomyrmecin is the main compound in Linepithema humile *ant venom*

Histological examination of the abdominal glands of *L. humile* and *T.* cf. *nigerrimum* workers confirmed that ants of both species have highly developed pygidial glands (Fig. 18a,b). We identified the different chemical compounds present in these pygidial glands via gas chromatography coupled with mass spectrometry (Fig. 18c,d).



Glands:
PyG, PyG-d: Pygidial gland and duct
PG,PG-r: Pavan gland and reservoir
VG, VGd: Venom gland and duct
DG: Dufour gland

Other structures:
GG: ganglion
HG: hindgut
OV: ovariole
VS: vestigial sting

Peak number in:		e
c	d	Compound
1	1	1-Decanol (IS)
	2	Iridodial 1
3		Dolichodial
	4	Tridecane
	5	Iridodial 2
	6	iridodial3
	7	Iridodial 4
8	8	Tetradecane
9	9	Iridomyrmecin 1
	10	Iridomyrmecin 2
	11	Dihydronepenta lactone
12		Pentadecane
	13	2-tridecanone
14		3-methyl pentadecane
15	15	Hexadecane

Fig. 18. Identification of *Linepithema humile* ant venom. Longitudinal section of the abdomen of (a) *L. humile* and (b) *T.* cf. *nigerrimum*. Partial chromatograms showing the iridodial/dolichodial iridomyrmecin complex of the pygidial glands of: (c) *L. humile* workers and (d) *T.* cf. *nigerrimum* workers. (e) List of compounds associated with the peaks in (c) and (d). Iridomyrmecin and iridodials with different numbers are isomers. Note that the hydrocarbons may have originated from the cuticular intima lining the gland (*Photo credits: Johan Billen*).

Our results show that iridomyrmecin (isomer 1) is the main compound found in *L. humile* pygidial glands (Fig. 18c,e). The major compounds found in the pygidial glands of *Tapinoma* species are isomers of iridodial, although iridomyrmecin is also present (isomers 1 and 2; Fig. 18d,e). Although *T.* cf. *nigerrimum* workers are slightly larger in body size than *L. humile* workers, *L. humile* contained five times more iridomyrmecin than *T.* cf. *nigerrimum* (mean ± SE: 6.416 ± 0.443 μg vs. 1.291 ± 1.127 μg; $F = 135.76$, $p < 0.0001$, $N = 100$). Iridomyrmecin represents 1.4% of worker fresh body mass in the invasive ant *L. humile* versus just 0.2% in the native ant *T.* cf. *nigerrimum*.

The functional ecology of iridomyrmecin

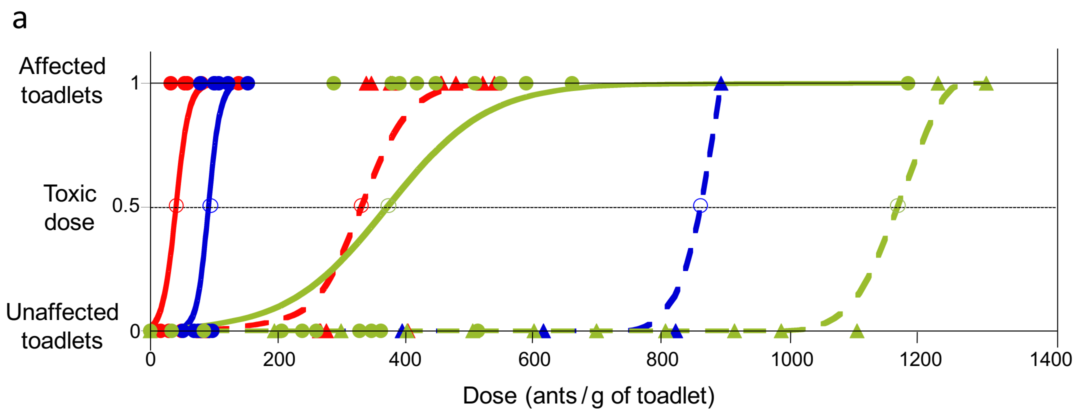
Our literature review on iridomyrmecin functional ecology unearthed 116 articles published between 1948 and 2018. When iridomyrmecin was assigned a function at its first mention in the text ($N = 93$), the two most frequently cited functions were “defense” (32%) and “insecticide” (32%) (Appendix 1a). Most of the ant species with iridomyrmecin belong to the Dolichoderine family and notably the genera *Iridomyrmex*, *Tapinoma*, and *Dolichoderus* (Appendix 1c). However, not all species in these genera have iridomyrmecin (e.g., *T. melanocephalum*, Tomalski *et al.* 1987). Iridomyrmecin has also been found in non-Dolichoderinae ants (i.e., *Pheidole biconstricta*, Davidson *et al.* 2005), in non-ant insects, (i.e. parasitic wasps and anthicide beetles, Appendix 1c) and in plants (Riddick *et al.* 2008). In all cases, iridomyrmecin has been reported to be an effective repellent. However, while Pavan and Ronchetti (1955) found that iridomyrmecin has insecticidal and antibiotic properties, they did not show that this compound was toxic for vertebrates (i.e., tests performed with dogs, rodents, and humans).

Iridomyrmecin-exposure experiments

To test iridomyrmecin’s toxicity, we synthesized and applied the compound to the backs of *P. cultripes* toadlets (isomers 1 and 2, in a ratio of 1.5:1). Three different doses were used: 0.1, 1, and 5 mg, which would correspond to an average dose ± SE of 8.4 ± 1.2, 69.7 ± 6.4, and 307.5 ± 30.3 *L. humile* workers per gram of toadlet, respectively. This calculation is based on the estimates described above and assumes that ants release all their pygidial gland contents at once. We observed significant differences among treatments ($\chi^2 = 25.63$, $p < 0.001$, $N = 42$). Seventy percent of the toadlets exposed to the highest dose displayed paralysis, what was significantly different from what was seen with the control ($p < 0.001$). The results for the lower doses were not significantly different from those for the control: all individuals remained alive at the end of the experiment ($p > 0.05$; Fig. 17c).

Toxic iridomyrmecin doses estimated for juvenile amphibians

To quantify the number of ants necessary to affect amphibians, we applied different numbers of mashed *L. humile* and *T.* cf. *nigerrimum* workers to juveniles of the three amphibian species. Generally, juveniles were increasingly affected by greater numbers of ants in a dose-dependent manner ($\chi^2 = 26.69$, $p < 0.001$, N = 81). However, the magnitude of the effect differed depending on both amphibian species and ant species ($\chi^2 = 23.40$, $p < 0.001$, N = 81 and $\chi^2 = 22.92$, $p < 0.001$, N = 81, respectively; Fig. 19a). Comparatively, smaller numbers of the invasive ant *L. humile* elicited more dramatic consequences than did larger numbers of the native ant *T.* cf. *nigerrimum* (Fig.19b).



b

TOXIC DOSE	Number of ants / g toadlet		mg iridomyrmecin / g toadlet	
	L. humile	*T.* cf. *nigerrimum*	*L. humile*	*T.* cf. *nigerrimum*
Epidalea calamita	40.1 ± 9.5 ━━━	328.0 ± 29.9 - - -	0.257 ± 0.061	0.423 ± 0.039
Pelobates cultripes	90.6 ± 6.5 ━━━	849.7 - - -	0.582 ± 0.042	1.097
Hyla meridionalis	367.8 ± 44.5 ━━━	1158.5 - - -	2.360 ± 0.285	1.496

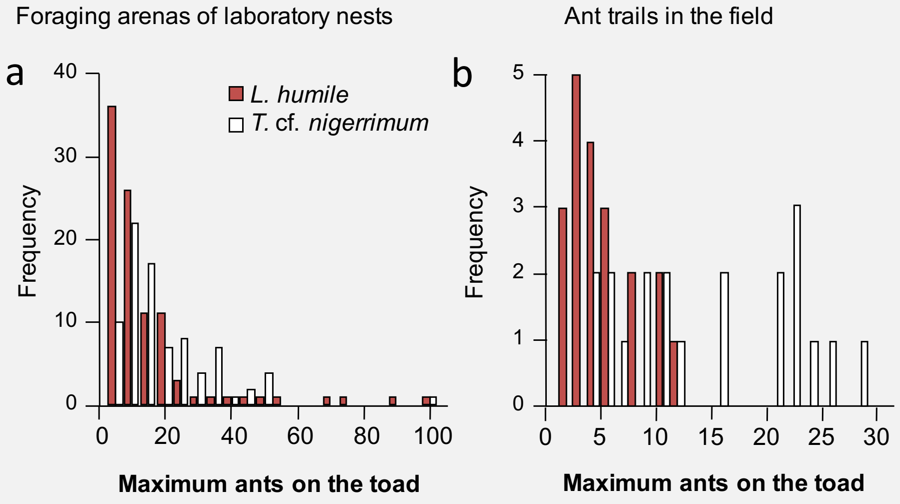
Fig. 19. Dose-response curves for three amphibian species (ant numbers per g of juvenile amphibian): *Epidalea calamita* (red), *Pelobates cultripes* (blue), and *Hyla meridionalis* (green). (a) Numbers of amphibians who were affected (1) or unaffected (0) (normal or abnormal reaction observed during clinical evaluation, see Methods) after spending 10 min in contact with a mash of different numbers of ants of the invasive *Linepithema humile* (straight lines/circles) or the native *Tapinoma* cf. *nigerrimum* (dashed lines/triangles). (b) Toxic dose, which was the mean number ± SE of ants (and the equivalent amount in mg ± SE of iridomyrmecin) per g of juvenile amphibian that elicited an effect. SE are only shown when meaningful. Equivalent amounts of iridomyrmecin were calculated using the species-specific contents: 6.416 ± 0.443 µg for *L. humile* and 1.291 ± 1.127 µg for *T.* cf. *nigerrimum*.

Box 3. Do toxic doses represent realistic numbers of ants relative to how many were seen on toads in lab and field experiments?

The mean ± SE of the maximum number of ants on the toad during the 10 min juveniles expended in the foraging arenas were 13 ± 2 and 17 ± 1.9 ants for *Linepithema humile* and *Tapinoma* cf. *nigerrimum* respectively, arriving in both cases to 100 ants. In the field, juveniles were covered by 5 ± 0.6 and 14 ± 1.7 *L. humile* and *T.* cf. *nigerrimum* coming from trails respectively. The activity of these trails, represented by the mean number of ants crossing a point per minute, was of 118.7 ± 14.5 and 125.8 ± 16.4 for *L.humile* and *T.* cf. *nigerrimum* respectively.

The toxic dose of the two toadlets was 40 (for *Epidalea calamita*) and 91 (for *Pelobates cultripes*) Argentine ants/g of juvenile, while the toxic dose of the froglet (*Hyla meridionalis*) was higher, 368 ants/g of juvenile. The mean mass (± SE) after metamorphosis in *E. calamita* was 0.45 ± 0.05 g, meaning that fewer than 20 *L. humile* workers could attack a toadlet and elicit an effect. Thus, the toxic doses for the Argentine ant (Fig. 19b) appear realistic and could cause paralysis and death in juvenile amphibians. Moreover, given the broad spatial extension of the *L. humile* nests, emerging *E. calamita* would likely have very low chances to survive in invaded areas (Fig 16b).

On the contrary, ant doses for the native ant *T.* cf. *nigerrimum* (Fig. 19b) were too high, especially for the more tolerant amphibian species; taking into account their low abundance and the reduced spatial extension of their nest, their impact seems to be limited.



c

Mean	SE	Max
13.0	2.0	99
17.9	1.9	100

d

Mean	SE	Max
5.1	0.7	12
14.0	1.7	27

Ants per minute in the trail

Mean	SE	Max
118.8	14.5	320
125.9	16.4	341

Maximum number of ants on the toad in the foraging arenas-exposure experiment and in the ant trail-exposure experiment: Frequency histograms (**a, b**) and mean ± SE, maximum number in the range (**c**, **d**). Ants per minute in the trails where the exposure was performed are also given in **d**, as an estimation of the ant activity.

Iridomyrmecin causes general paralysis and histological lesions

We observed that the venom of the invasive ant *L. humile* mainly had neurological consequences, specifically in the medulla oblongata, pontine nucleus, and midbrain. The principal signs were general paralysis (Fig. 20a), which could be accompanied by the loss of photopupillary and palpebral reflexes and the loss of the nociception response. We also observed severe damage to the skin of juveniles that came in contact with *L. humile* and of juveniles treated with iridomyrmecin (Fig. 20b).

Individuals showing signs of neurological damage had higher levels of iridomyrmecin in their brains than did unaffected individuals ($\chi^2 = 10.19$, $p = 0.001$, $N = 28$). Moreover, concentrations of iridomyrmecin in brain, liver, and kidney tissue were significantly correlated with the equivalent amounts of iridomyrmecin applied (brain: $F = 17.69$, $p < 0.001$, $N = 28$; liver: $F = 14.24$, $p < 0.001$, $N = 27$; kidney: $F = 8.29$, $p = 0.008$, $N = 26$; Fig. 20c).

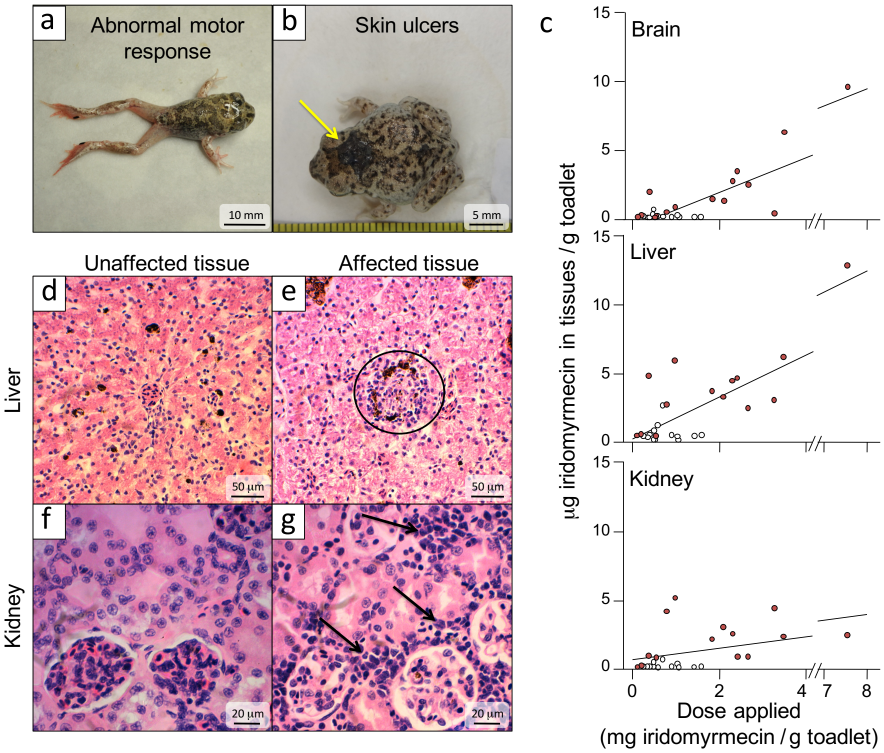


Fig. 20. Effects of iridomyrmecin on toadlets and toadlet tissues. *Pelobates cultripes* toadlet with (a) an abnormal motor response (ataxia, paresis, and flaccidity) and with (b) skin ulcers (marked with an arrow). (c) Relationship between the dose applied (equivalent amounts of iridomyrmecin estimated from the number of *Linepithema humile* [in red] or *Tapinoma* cf. *nigerrimum* [in white] applied to toadlets) and the concentration of iridomyrmecin measured in toadlet tissues after treatment. Model fit was determined using the combined data for all the amphibian species and ant species. (d) Unaffected liver. (e) Damaged liver with lymphoplasmocytic inflammatory infiltrates in the periportal space (circled). (f) Unaffected kidney. (g) Damaged kidney with acute tubulo-interstitial nephritis (marked with arrows) (*Photo credits: Paloma Alvarez-Blanco and Alejandro Bertó-Morán*).

The histological samples revealed liver and kidney damage had occurred, indicating the toxin's acute effects on these tissues. In the liver, we found inflammatory cell infiltrates (heterophils) around the hepatic artery (Fig. 20d,e). These lesions were observed in 16 cases (N = 33, all species combined). There was no significant relationship between the quantity of iridomyrmecin per gram of amphibian and the presence of lesions ($\chi^2 = 0.12$, $p = 0.727$, $N = 33$), which could be due to the individuals' short exposure to the toxin (only 10 min; see Methods). In the kidney, we found inflammatory cell infiltrates (lympho-plasmocitary cells) in the renal tubules, which indicated tubulo-interstitial nephritis (Fig. 20f,g). We observed lesions in just five cases (N = 32, all species combined). Range of doses administrated were 0.208 to 0.885 mg of iridomyrmecin per gram of amphibian for *E. calamita*; 0 to 0.796 mg for *P. cultripes* and 0 to 3.766 mg for *H. meridionalis*. Lesions were found in individuals who had received mean doses of 0.674, 0.665, and 1.167 mg of iridomyrmecin per gram of amphibian for *E. calamita*, *P. cultripes*, and *H. meridionalis*, respectively.

Potential global impacts on amphibians

We examined the overlap across the globe between the distribution ranges of amphibian species and the locations of 1,407 *L. humile* populations, 61 of which were native, while the rest were invasive (Appendix in Digital CSIC: http://hdl.handle.net/10261/173421). There were only 51 *L. humile* populations (all invasive) that were not associated with any amphibians.

Using the full dataset, we determined that, worldwide, *L. humile* populations potentailly co-occurred with a total of 813 amphibian species (based on the 6,513 terrestrial amphibian species with spatial data in the IUCN Red List database), and only 9 of these amphibians exclusively co-occurred with native *L. humile* populations. Outside of its native range, *L. humile* potentially co-occurs with a mean (± SE) of 11.06 (± 0.23) amphibian species per locality (range: 1–86, N = 1295; Fig. 21).

After filtering the amphibian species based on microhabitat, we found that *L. humile* populations potentially co-occurred with a total of 693 amphibian species. Outside of its native range, *L. humile* potentially co-occurs with a mean (±SE) of 7.22 (± 0.20) amphibian species per locality (range: 1–78, N = 1287; Appendix in Digital CSIC: http://hdl.handle.net/10261/173421).

At the global scale, between 6.27% (full dataset) and 5.48% (microhabitat-filtered dataset) of these amphibian species fell into one of the three IUCN Red List categories for threatened species: 8–9 were critically endangered (CR), 10–16 were endangered (EN), and 20–26 were vulnerable (VU), depending on the dataset used. In contrast, between 91.39% (full dataset) and 92.21% (microhabitat-filtered dataset) of the amphibian species were in the least threatened categories: 34–42 were near threatened (NT) and 701–605 were of least concern (LC). The remaining 2.34% (full dataset; 19 species) and 2.31% (microhabitat-filtered dataset; 16 species) were listed as data deficient (DD). At the regional scale, within the native range of the Argentine ant, only 2.50–2.74% of these amphibian species are threatened (CR, EN, VU). In contrast, in the rest of the world, these percentages are notably higher: 3.17–3.49% in the rest of South America, 5.41–6.47% in North America, 7.69–8.57% in Europe, 5.07–5.73% in Africa, and as high as 13.21–16.39% in Australia (Fig. 21). The impact of the Argentine ant could be particularly serious in Sub-Saharan Africa, as this region has the largest number of potentially affected critically endangered amphibian species (Fig. 21).

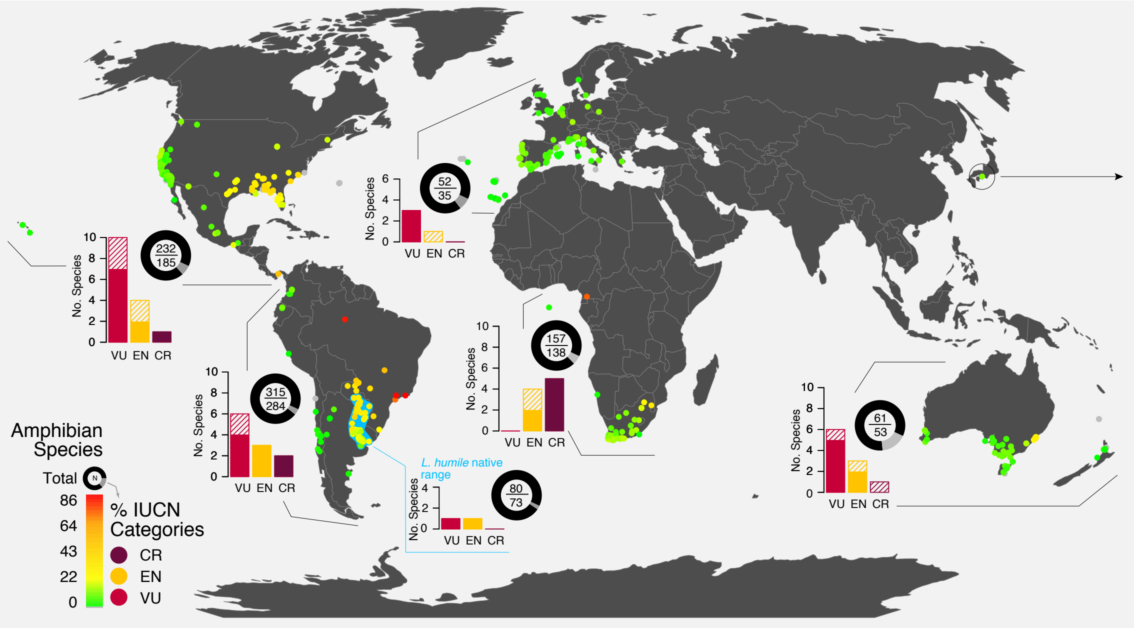


Fig. 21. World map showing records of native and invasive *Linepithema humile* populations (native range in light blue). Point color reflects the number of co-occurring amphibian species (1: green to 86: red; 0: gray) based on spatial and macrohabitat overlap. The following regions were defined: the native range of *L. humile*, the rest of South America, North America (plus Japan), Europe (including European island colonies in North Africa), Sub-Saharan Africa, and Oceania. The pie charts show regional species richness (range of cumulative number of species for the full dataset [top] and for the microhabitat-filtered dataset [bottom]) and the proportion of species in non-threatened (black) and threatened (gray) IUCN Red List categories (for the full dataset). The bar charts for each region show the number of species that are vulnerable (VU), endangered (EN), and critically endangered (CR) for both the full (hashed) and microhabitat-filtered (filled) datasets.

Discussion

We discovered that iridomyrmecin can kill juveniles of terrestrial amphibians. Understanding the mechanisms that underlie the impacts of invasive species helps scientists assess the potential magnitude of those impacts, which is essential when prioritizing and managing invasions, as made clear in the Aichi targets of the Convention of Biological Diversity (Strategic Plan 2020-Aichi targets, target 9). In contrast to previously described impacts of invasive species (Kumschick *et al.* 2015), this newly discovered effect of *L. humile* is context independent: ants are a common prey for anurans (Wells 2010), and *L. humile* is an invasive ant species worldwide that displaces native ants and attains high population densities (Angulo *et al.* 2011; Holway *et al.* 2002). Our results reveal that *L. humile* invasions may have global consequences—the Argentine ant is a potential threat to populations of terrestrial amphibians, an already endangered vertebrate taxon (Bosch *et al.* 2018; Duarte *et al.* 2012; Hoffmann *et al.* 2010). A total of 813 amphibian species around the world overlap in range and macrohabitat with the Argentine ant and could therefore be affected by the species' aggressive behavior and chemical weaponry. Of these species, 6.27% are classified as threatened by IUCN (i.e., critically endangered, endangered, or vulnerable). At the regional level, this percentage is as high as 16.39% (in Australia).

The invasive ant *L. humile* has had striking success in Mediterranean ecosystems across almost all continents (Suarez *et al.* 2001; Vogel *et al.* 2010). It excludes native ant species from communities and forms colonies that spread over enormous areas (Giraud *et al.* 2002), which are then completely saturated with groups of nests and intertwined trails (Angulo *et al.* 2011; Heller *et al.* 2008). Native amphibians in Doñana were found to avoid favorable habitats that had been colonized by *L. humile*, likely because the invasive ant was not desirable prey (Alvarez-Blanco *et al.* 2017 = Section 1). A similar phenomenon might be responsible for the decline of another ant predator, the coastal horned lizard in California (Suarez and Case 2002). However, in light of our results, the decline of these native predators could be also due to reduced juvenile survival in invaded areas. Attention should also be paid to other juvenile amphibians known to consume Argentine ants in invaded areas (Beard 2007; Ito *et al.* 2009).

Pavan and Ronchetti (1955) concluded that iridomyrmecin toxicity was very low for homoeothermic species but that the compound could be toxic for non-homoeothermic species. Here, we clearly demonstrate that iridomyrmecin is toxic for amphibians when topically applied (its toxicity upon ingestion is still unknown). However, given reports that *L. humile* also preys on birds (Hooper-Bui *et al.* 2004; Sockman 1997; Suarez *et al.* 2005), Pavan and Ronchetti's assertion seems to be lacking. Given the venom's toxicity together with the ecological dominance of *L. humile*, it is very likely that iridomyrmecin has important ecological implications for natural populations of amphibian species. However, more research is needed to explore this subject and to examine the factors underlying venom toxicity in other taxa (e.g., skin permeability or life-history traits, such as developmental type or breeding strategy).

In its native range *L. humile* does not dominate ant communities (Holway and Suarez 2004; Suarez *et al.* 1999) and its nests are several orders of magnitude smaller than in its invasive range (Holway *et al.* 1998; Pedersen *et al.* 2006). Thus, in the native range, even if the venom causes harm to co-occurring amphibians, the overall impacts are likely limited by the species' relative low ecological dominance.

Iridomyrmecin is not specific to *L. humile* (Hefetz and Lloyd 1983; Wheeler *et al.* 1977). However, we demonstrated that its quantities in the native species *T.* cf. *nigerrimum* are too low to affect native juvenile amphibians. Although iridomyrmecin is also present in other ant species (e.g., Attygalle and Morgan 1984; see Appendix 1), the information available do not allow us to establish a link between the presence of iridomyrmecin and invasiveness; e.g. it is not present in another invasive Dolichoderinae ant, *T. melanocephalum* (GISD; Tomalski *et al.* 1987). However, the convergent occurrence of this compound in different insect families and orders indicate its effectiveness as a repellent. Moreover, the iridomyrmecin-exposure experiment demonstrated that iridomyrmecin is the active substance. Other pygidial gland compounds besides iridomyrmecin may also function as defensive compounds (e.g., dolichodial, Cavill *et al.* 1976; Welzel *et al.* 2018) or help the venom to reach and stay on the target (e.g., iridodials, Attygalle and Morgan 1984). Differences in such compounds could explain the differences we found in the toxic dose of iridomyrmecin for a given amphibian species when using *L. humile* versus *T.* cf *nigerrimum* (Fig. 19b).

Our results showed that the venom caused general paralysis, which occurred rather quickly following initial contact, and indicated intra- and interspecific differences in tolerance among amphibians. The most tolerant species, the frog *H. meridionalis*, could escape from ant trails in the field even after two minutes of contact, but more subtle effects were observed when the species was confined for a longer time period. These results suggest that jumping behavior could favor a quicker escape. In fact, juveniles of *H. japonica* have been found to consume Argentine ants without seeming to experience any negative impacts (although the researchers did not look for them; Ito *et al.* 2009). The dose-response experiment confirmed lower tolerance for *E. calamita* and *P. cultripes* toadlets, and indicated differences in the toxic doses of the invasive versus the native ants. Taken together, these results explain why *L. humile* had a detrimental effect on small toadlets, such as those of *E. calamita*. In this species, the mean mass (± SE) after metamorphosis was 0.45 ± 0.05 g, meaning that fewer than 20 *L. humile* workers could attack a toadlet and elicit an effect; in contrast, more than 150 *T.* cf. *nigerrimum* workers would be required (Fig. 19b). Although *T.* cf. *nigerrimum* was more efficient than *L. humile* in recruiting to the juveniles, the greater threat may come from *L. humile*, given its greater toxicity and its high abundance around invaded ponds (Figure 16a,b; Alvarez-Blanco *et al.* 2017 = Section 1; Angulo *et al.* 2011). Emerging *E. calamita* would likely have slim chances of surviving in invaded areas. Further research to accurately estimate how much iridomyrmecin ants release during attacks and to characterize iridomyrmecin variability (e.g., over time and space) is necessary if we wish to improve estimates of toxic doses for amphibian species.

Although *L. humile* is one of the best-studied invasive ant species (Sanders and Suarez 2011), its use of the venom iridomyrmecin as a tool for preying on amphibians has been overlooked. The red imported fire ant, *S. invicta*, impacts native herpetofauna, birds, and mammals (Allen *et al.* 2017; Allen *et al.* 2004). Its venom accumulates in the poison gland and consists of alkaloids and a small amount of proteins. Although it is normally injected by stinging, it can also be sprayed (Liu *et al.* 2017). The venom induces anaphylaxis (causing cardiac and respiratory problems) or paralysis and death at higher doses (Langkilde *et al.* 2017). In contrast, research on the little fire ant, *W. auropunctata*, and the yellow crazy ant, *A. gracilipes,* is scarcer (Sanders and Suarez 2011). Most reports on the impacts of *W. auropunctata* on vertebrates have been anecdotal, with no evidence that predation is occurring (Jourdan *et al.* 2001; Wetterer and Porter 2003). Like *S. invicta*, *W. auropunctata* has a painful stinger that induces anaphylaxis. By comparison, *A. gracilipes* sprays formic acid—an irritant located in the poison gland—to subdue prey (Bertelsmeier *et al.* 2016); however, evidence that the ant preys on vertebrates remains sporadic (Davis *et al.* 2008; Matsui *et al.* 2009; Plentovich *et al.* 2018).

The role of *L. humile* as a predator is not apparent, which is likely why it has remained unstudied. First, the species does not possess a functional stinger. Second, the venom does not appear to affect humans or mammals (Pavan and Ronchetti 1955). Third, amphibians could not become immediately paralyzed but die later and thus there is no obvious link tying the death to ants. Indeed, this species is mainly considered to be a scavenger (Angulo *et al.* 2011) that consumes a carbohydrate-rich diet fueled by the species' mutualism with aphids (Rowles and Silverman 2009). Moreover, reports of occasional predation (Table 1) have been crowded out by research focusing on unicoloniality, a trait that has elicited substantial scientific attention (Giraud *et al.* 2002; Vogel *et al.* 2010).

This study reveals that the invasive ant *L. humile* can prey on vertebrates. Furthermore, it describes the proximate mechanisms (behavioral, chemical, and histological) that cause amphibian mortality. Additionally, we underscore that this invasive species may have global impacts, given its worldwide overlap with amphibian species, many of which are already threatened by other factors (Bellard and Jeschke 2016; Murray *et al.* 2014). Invasion biology examines invasion causes and consequences and specifically focuses on ecosystem-level impacts. The goal is to provide adequate tools for eradicating, or at least mitigating, the impact of current invasions (Blackburn *et al.* 2014; Kumschick *et al.* 2015). Our study stresses that the continued acquisition of knowledge and the questioning of assumptions is crucial in this task. We also highlight the need for new research to more fully clarify the impacts of the most invasive species; for example, global networks may be key (Packer *et al.* 2017).

Section 3: Survival and growth of native toadlets feeding on an invasive ant prey

Results

Experiment 1. Raising metamorphic toadlets on invasive or native ants

Pelobates cultripes toadlets showed low mortality rates throughout the experiment, and did not vary between those that received the native or the invasive ant prey ($\chi^2 = 0.55$, $p = 0.552$, N = 52, Fig. 22a). By contrast, *Epidalea calamita* toadlets showed significantly higher mortality rates when they were supplemented with the invasive ant than with the native ant ($\chi^2 = 4.32$, $p = 0.038$, N = 96; Fig. 22b).

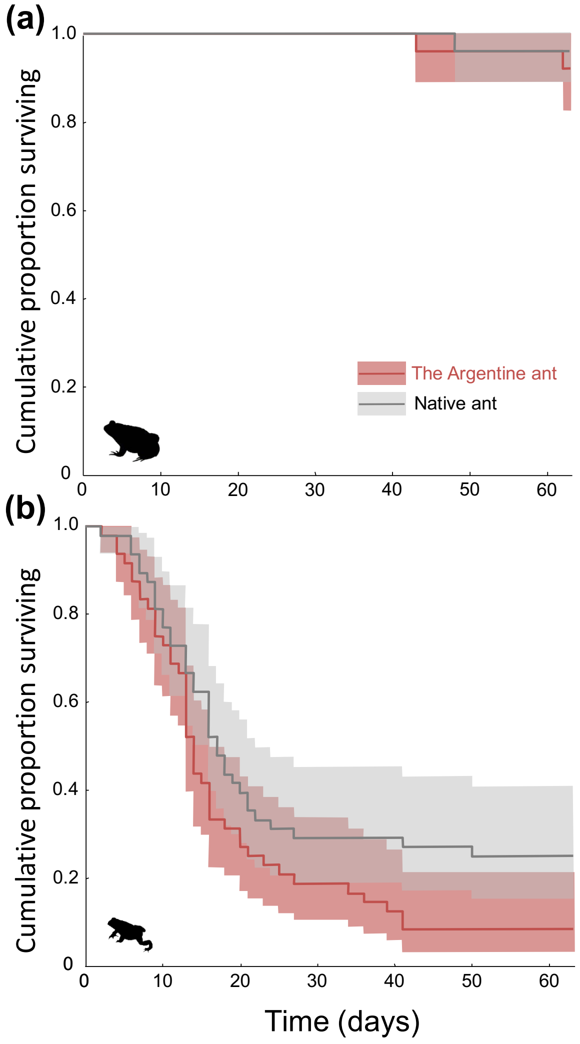


Fig. 22. Survival of *Pelobates cultripes* (a) and *Epidalea calamita* (b) toadlets raised on a diet supplemented with the invasive Argentine ant *Linepithema humile* (red) or the native ant *Tapinoma* cf. *nigerrimum* (grey). Shaded areas represent 95 CI.

Toadlets' ***weight*** increased considerably over the experiment irrespective of treatment (Fig. 23a,b). The increase was from 780 ± 13 to 1,853 ± 45 mg (mean ± SE, N = 49) for *P. cultripes*, an average 2.38 – fold increase, whereas it was changed from 77 ± 3 to 161 ± 8 (mean ± SE, N = 16) for *E. calamita*, an average 2.09 – fold increase. In both species, the interaction of treatment with time was significant (LRT = 26.63, $p < 0.0001$, N = 49 and LRT = 4.24, $p = 0.039$, N = 16, for *P. cultripes* and *E. calamita* respectively) indicating that growth rate differed between treatments. Toadlets in the invasive ant treatment grew significantly less along the experiment (Fig. 23a,b).

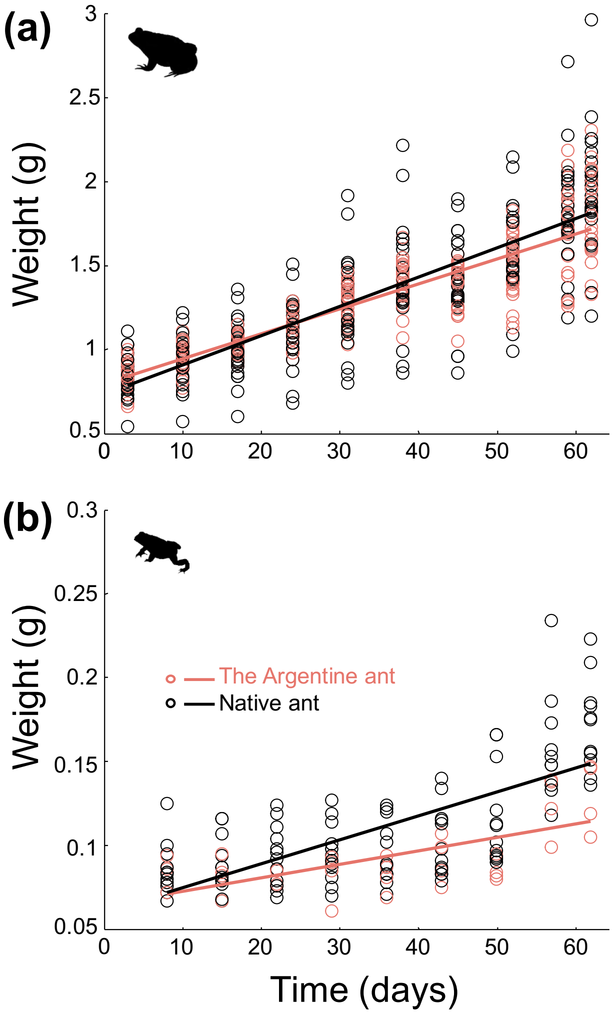


Fig. 23. Growth of *Pelobates cultripes* (a) and *Epidalea calamita* (b) toadlets raised on a diet supplemented with the invasive Argentine ant *Linepithema humile* (red) or the native ant *Tapinoma* cf. *nigerrimum* (black).

Regarding food intake, the proportion of ***crickets consumed*** increased over time (Fig. 24a,b) and varied significantly between treatments (LRT = 7.44, p = 0.006, N = 49, and LRT = 4.62, p = 0.032, N = 16, for *P. cultripes* and *E. calamita* respectively). More importantly, the interaction between time and treatment was significant, meaning that the proportion of crickets toadlets ingested throughout the experiment increased less in the invasive ant treatment than in the treatment with the native ant (LRT = 30.89, p < 0.001, N = 49, and LRT = 19.757, p < 0.001, N = 16, for *P. cultripes* and *E. calamita* respectively).

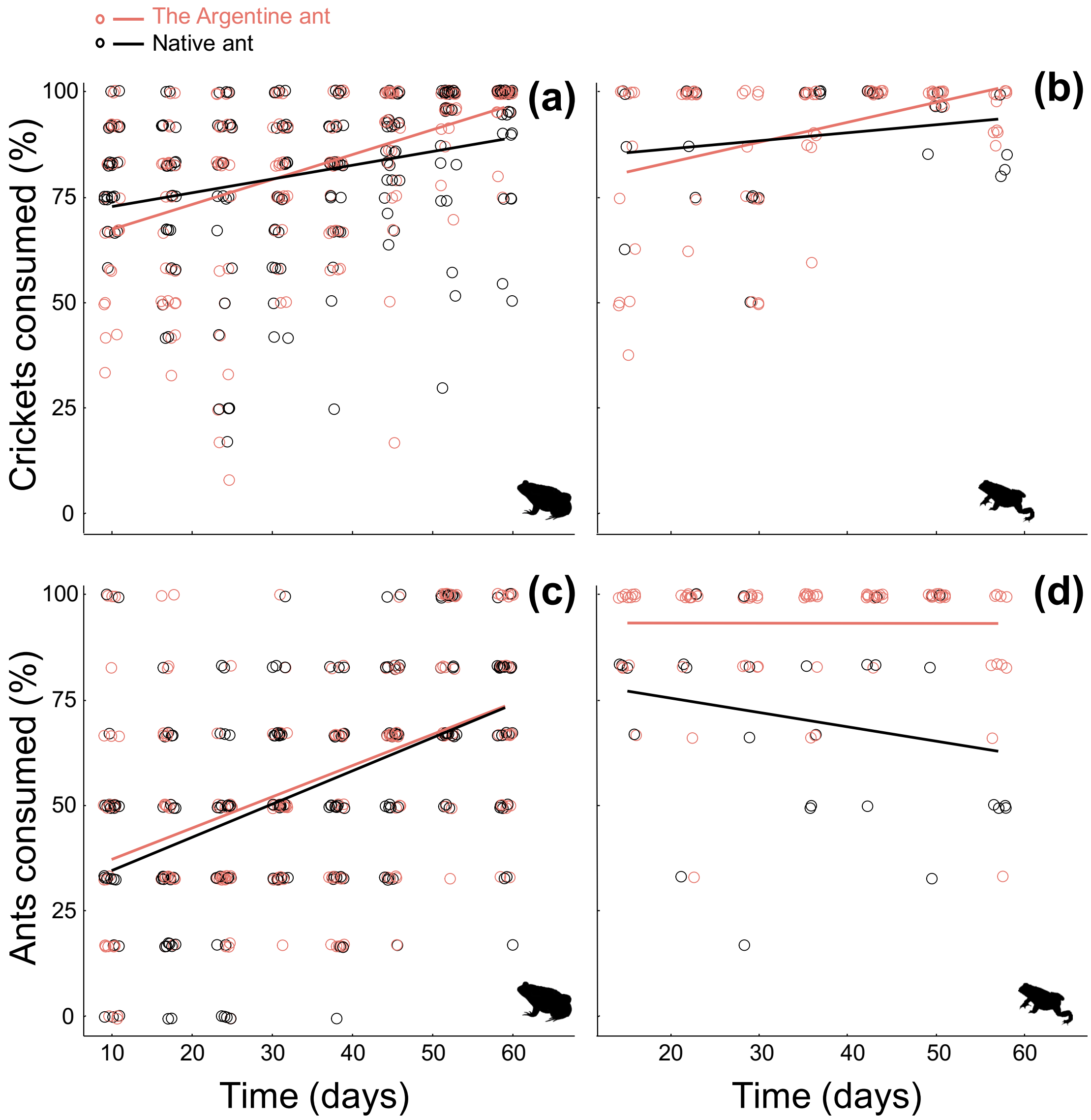


Fig. 24. Food intake of *Pelobates cultripes* (a, c) and *Epidalea calamita* (b, d) toadlets raised on a diet supplemented with the invasive Argentine ant *Linepithema humile* (red) or the native ant *Tapinoma* cf. *nigerrimum* (black). a,b: Crickets consumed ; c,d: Ants consumed. To avoid over plotting, points are slightly displaced.

Ant intake did not vary significantly between treatments over time (LRT = 0.40, p = 0.530, N = 49 and LRT = 0.44, p = 0.508, N = 16, for *P. cultripes* and *E. calamita* respectively). Ant intake increased over time for *P. cultripes* but not for *E. calamita* (Fig. 24c,d), even though the number of ants offered was constant throughout the experiment. Moreover, there were significant differences between treatments for *E. calamita* but not for *P. cultripes* (LRT = 8.04, p = 0.005, N = 16 and LRT = 0.24, p = 0.625, N = 49, respectively). Ant intake in *E. calamita* was lower in the invasive ant treatment than in the native ant treatment (Fig. 24d).

Foraging efficiency of *P. cultripes* toadlets after the two-month experiment was not significantly different between treatments (Table 5). Neither the mean time to prey discovery, nor the motivation or the accuracy differed significantly between treatments (F = 0.004, p = 0.950; LRT = 0.04, p = 0.845; and LRT = 0.693, p = 0.405, respectively, N = 35, Table 5). Sample size for *P. cultripes* was reduced to 35 toadlets because 11 failed to recognize the prey (six in the invasive ant treatment and five in the native ant treatment). Although time to prey discovery was not significantly different between treatments for *E. calamita* toadlets (LRT = 0.91, p = 0.339, N = 16), motivation and accuracy differed significantly between them (LRT = 7.50, p = 0.006, and LRT = 4.73, p = 0.030, respectively, N = 16, Table 5). Motivation (the number of attempts) was higher in the treatment with the native ant than in the invasive ant treatment, while the accuracy (the success in prey capture) was higher in the invasive ant treatment than in the native ant treatment. Sample size for *E. calamita* toadlets corresponds to 16 individuals that performed the first test, but four were missing in the second test.

Table 5. **Foraging efficiency.** Time to prey discovery (discovery), number of attempts to prey (motivation), and prey capture success (accuracy) of toadlets fed during two months with a diet supplemented with the invasive Argentine ant or a native ant species. The number of individuals for *Pelobates cultripes* or the number of repetitions for *Epidalea calamita* is given in brackets. Significant differences are marked in bold (**p<0.01, *p<0.05).

	Pelobates cultripes		*Epidalea calamita*	
	Argentine ant	Native ant	Argentine ant	Native ant
Discovery (s)	45 ± 11 (17)	49 ± 15 (18)	19 ± 6 (7)	15 ± 6 (21)
Motivation	3 ± 1 (17)	3 (18)	**3 ± 1 (7)****	**10 ± 2 (21)****
Accuracy (%)	62 ± 9 (17)	71 ± 6 (18)	**63 ± 14 (7)***	**39 ± 5 (21)***

All *E. calamita* toadlets in the invasive Argentine ant treatment (N = 4) had visible ***ulcers on the skin*** at the end of the experiment (Fig. 25). Ulcers were detected in none of the *E. calamita* toadlets in the native ant treatment (N = 12), neither on any *P. cultripes* toadlets from any treatment (N = 49).

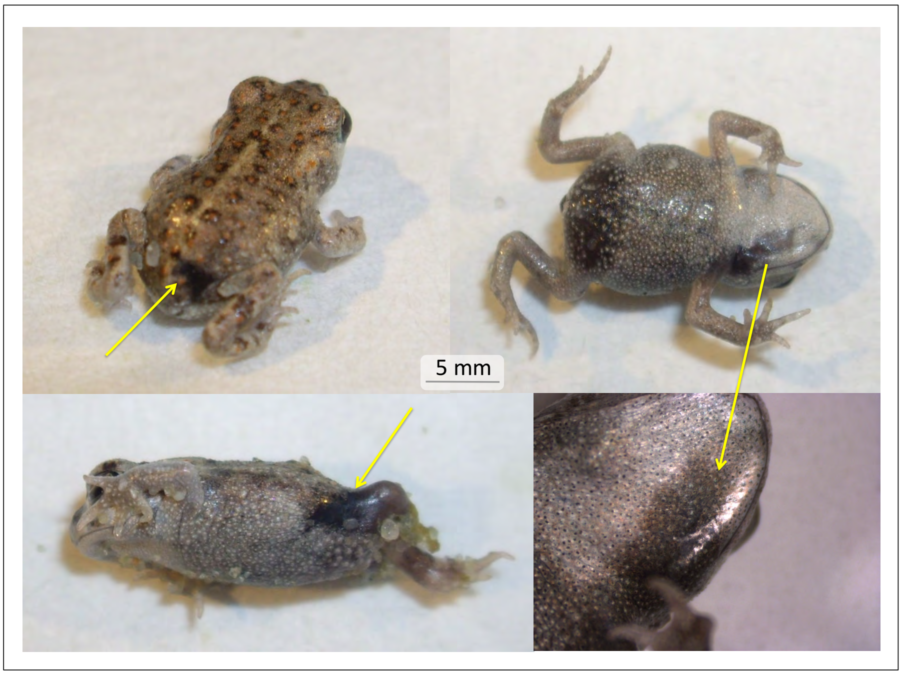


Fig. 25. Ulcers on the skin found on *Epidalea calamita* toadlets after exposure to invasive Argentine ants (yellow arrows) (*Photo credits: Paloma Alvarez-Blanco*).

Experiment 2. Feeding on invasive or native ants

***Weight** of E. calamita* toadlets increased considerably over the one-month experiment, from 412 ± 15 to 715 ± 20 mg (mean ± SE, N = 15, Fig. 26), an average 1.73 – fold increase. The interaction between time and treatment was significant (LRT = 10.00, p=0.007, N = 15), and differences were driven by a lower increase in toadlet growth in the native ant treatment than in the treatment with no ants (t = 3.19, p = 0.007, Tukey test). Nevertheless, there were no significant differences in toadlet growth between the invasive ant and the native ant treatments or between the invasive ant treatment and the treatment without ants (Tukey test: t = 1.21, p = 0.453; t = 1.98, p = 0.127, respectively).

With respect to the ***digestive lesions in toadlets***, there were no significant differences among treatments, neither in the presence of middle term lesions, nor in their size (LRT = 0.29, p = 0.863, N = 15; F = 1.17, p = 0.351, N = 13, respectively).

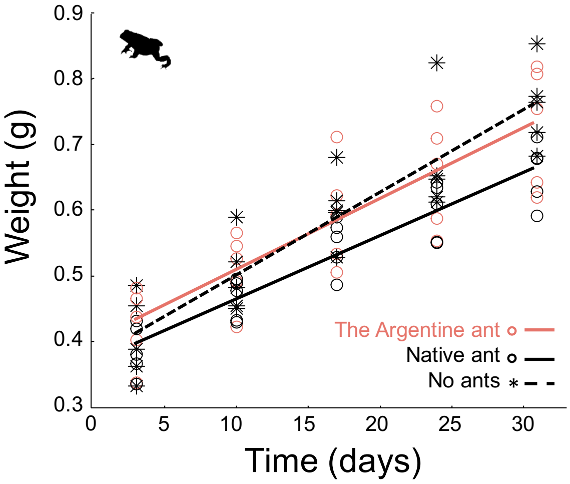


Fig. 26. Growth of *Epidalea calamita* toadlets on a diet of crickets but manually supplemented with either the invasive Argentine ant *Linepithema humile* (red) or the native ant *Tapinoma* cf. *nigerrimum* (black circles, black solid line). Control toadlets were also manually fed crickets (black asterisks, black dashed line) to control for handling stress.

Discussion

Invasive prey species can affect predators within invaded ecosystems, reducing their individual fitness, with potential consequences at the population level. We predicted that survival and growth would decrease when amphibians consumed invasive Argentine ants, and we found that to be the case, although the effects differed between the amphibian species studied. Toadlets of *E. calamita* showed increased mortality and decreased growth when feeding on the invasive Argentine ant compared to toadlets feeding on the native ant species (Fig. 22b, 23b). In turn, *P. cultripes* toadlets fed on Argentine ants survived well but showed decreased growth rates (Fig. 22a, 23a).

Most of our notion of the impact of the Argentine ant on other species focuses on population effects, such as displacement of native ants, decrease in population abundance of arthropods, or population declines in predators (Holway *et al.* 2002). However, the mechanisms by which Argentine ants impact native predators have been less studied. In the horned lizard, Suarez and Case (2002) showed a reduction in growth when this lizard had a diet consisting of Argentine ants. Our results also confirm lower growth rates for the newly metamorphosed toadlets of two amphibian species. Suarez and Case (2002) explained that this reduction was a consequence of their lower preference for the Argentine ant because of their smaller size, possible unpalatability due to novel defensive compounds, and aggressive behavior (Suarez *et al.* 2000). Alvarez-Blanco *et al.* (2017 = Section 1) also showed lower preference for Argentine ants than for native ants in adults of *E. calamita*, probably due to the small size and color of the ants. This is in agreement with the lower Argentine ant consumption compared to native ants by *E. calamita* juveniles observed in this study. Alvarez-Blanco *et al.* (2017 = Section 1) also showed that *E. calamita* toadlets captured in areas invaded by the Argentine ant fed on a great amount of these ants . However, *P. cultripes* toadlets in our experiment consumed both ant species equally, increasing their consumption as the experiment progressed. Alvarez-Blanco *et al.* (2017 = Section 1) showed that adults of *E. calamita* also increased the ant intake over trial repetition, with either native or invasive ants, indicative of a learning process.

Robbins *et al.* (2013) suggested that the eastern fence lizard (*Sceloporus undulatus*) learned to feed on the red imported fire ant (*Solenopsis invicta*) as a mechanism to avoid higher external envenomation due to ant recruitment. However, in order to get protection from the defensive compounds of the Argentine ant, toads have the ability to bury in the sand, as we observed. Overall lower consumption of Argentine ants by *E. calamita* toadlets in our experiment could also be interpreted as a strategy to avoid external envenomation. The Argentine ant has been recently discovered to be able to kill toadlets with their defensive compounds, a lethal venom that is absorbed through the toadlets' permeable skin (Section 2). Moreover, toadlets in the Argentine ant treatment ate lower amounts of crickets, which was their main food source. This lower cricket consumption could explain their reduced growth rate, and be due to prolonged periods of time spent buried and consequently reduced foraging time in individuals exposed to the Argentine ant. Behavioral alterations in the presence of invasive ants have also been shown in southern toads (*Anaxyrus terrestris*), which increased the number of movements in the presence of the red imported fire ant (Long *et al.* 2015). The eastern fence lizard also altered their behavior in the presence of the red imported fire ant (Langkilde 2009). Although such behavioral alterations may lessen the direct effects via predation and injury, they have been suggested to increase energy expenditure in order to support the movement itself or by losing feeding opportunities (Langkilde *et al.* 2017).

Toadlets of *E. calamita* raised on invasive Argentine ants showed higher mortality than those fed with the native ant (Fig. 22b). However, when we isolated ant consumption from simple external contact in the second experiment, survival and growth rates did not differ between toadlets consuming Argentine ants or native ants.

This difference in survival indicated that the problem was not the ingestion of ants *per se* but the presence of ants in the terraria, that implies the need to detect and capture the ants to feed on them and allows their external physical contact with the toadlets. Increased mortality could have been due to reduced ingestion of Argentine ant if these were harder to detect being smaller and less shiny than the native one (Alvarez-Blanco *et al.* 2017 = Section 1; Suarez *et al.* 2000). However, the main source of food for the toadlets was crickets, so reduced ant ingestion should not have been a life-threatening problem itself. Second, the presence of defensive chemical compounds and high aggressiveness has been suggested as explanation for the lower preference for the Argentine ant by predators (Suarez and Case 2002; Suarez *et al.* 2000). In this sense, the presence of ulcers on the skin of *E. calamita* toadlets surviving the invasive ant treatment, suggested the Argentine ant attacked them chemically. Even though we took into consideration offering only two ants at once to avoid topic envenomation, we were not able to prevent it completely. Moreover, although *E. calamita* toadlets are capable of burying when conditions are unfavorable (Gómez-Mestre and Tejedo 2005) we frequently observed in our experiments that they were only partially covered in the hole they dug, and we observed ants having access to them. These differences in the burying behavior could also contribute to explain differences in survival rates between amphibian species.

Moreover, the venom of the Argentine ant does not equally affect both species (Section 2). When the toxic compounds of the Argentine ant were applied topically, *E.calamita* toadlets showed envenomation at lower doses (40 ants/g of toadlet) than *P.cultripes* toadlets (90 ants/g of toadlet) (Section 2). Both amphibian species also differ in their life history, with *E. calamita* having a shorter larval period than *P. cultripes*, which is translated into much smaller toadlets at metamorphosis. *Epidalea calamita* toadlets were eleven-fold smaller than those of *P. cultripes* at the beginning of our experiment (weight (mean ± SD): 67 ± 12 mg (N = 96); 778 ± 91 mg (N = 52), respectively). It is unknown whether the toxin could have an accumulated and potentiated effect over time. If we translate the lethal ant dose (40 ants/g of toadlet) into our experiment, *E. calamita* toadlets would have received the lethal dose after about three weeks, but mortality was continuous from the beginning of the experiment. All this information combines to indicate that the most likely reason for the decrease in *E.calamita* survival when raised on Argentine ants is their use of their defensive compounds against the predator.

The surviving *E. calamita* toadlets from the Argentine ant treatment showed a lower motivation (lower number of attempts) but a higher accuracy (higher prey capture success) when they decided to capture a cricket, compared to those surviving the native ant treatment. Native species that do not appropriately respond to invaders can adapt over time, through differential survival of individuals showing the correct responses. For example, fence lizards occupying invaded areas by the fire ant showed specific behavioral strategies (e.g., increase in twitch and flee behavior following ant detection; Freidenfelds *et al.* 2012; Langkilde 2009). In our case, toadlets of better foraging efficiency were the ones that survived the Argentine ant treatment, probably because better foraging efficiency in this case reduced the odds of topical envenomation.

Contrary to our predictions, we did not find evidence for lesions in the digestive system (i.e. stomach and gut) caused by the venom of the Argentine ant in *E. calamita* toadlets consuming them. *Epidalea calamita* is a common myrmecophagous species, and this result raises the question of whether the venom present in the Argentine ant (Pavan 1952d, Section 2) could have been neutralized during the digestion process in this species. For example, the horned lizard is able to detoxify the venom when feeding on a toxic native ant (*Pogonomyrmex*) (Schmidt *et al.* 1989). Although the venom of the Argentine ant was novel for the native toadlets, amphibians are known to feed on numerous prey items with a variety of toxic substances, and some have evolved chemical defenses to neutralize those toxins and even capitalize on them to become poisonous themselves (Caldwell 1996; Saporito *et al.* 2012). Up until now, the toxicity of the Argentine ant has only been tested via topical route, when applied on the skin of amphibians (Section 2). Here, we test for the first time the toxicity of the Argentine ant via oral route and did not find any deleterious effect, suggesting that toads could be able to detoxify iridomyrmecin in their digestive track.

The presence of the Argentine ant and feeding on it may reduce the survival and slow down the development of native amphibians in their most vulnerable terrestrial stages, when they are juveniles. The early stages of the life-cycle of an organism are crucial and determine their future survival (Pechenik 2006; Wu *et al.* 2012). Our results highlight a new danger imposed by an invasive species to already endangered taxa. The negative bottom-up effects of the Argentine ant invasion to native predators need to be incorporated to the list of previously reported ecosystem impacts.

Section 4: The physiological consequences of growing in Argentine ant invaded areas

Results

Ant attraction to artificial avian nests

While in invaded areas only the Argentine ant was present at the artificial nests, four native ants were found visiting the nests in uninvaded areas: *Crematogaster scutellaris*, which was the most common, followed by *Lasius grandis*, *Formica subrufa*, and *Camponotus lateralis*. The maximum number of ants visiting the artificial nests was 190 native ants in uninvaded areas and 350 Argentine ants in invaded areas. Temperatures recorded ranged between 17 and 31 ºC, typical of the season in the area.

Normal ant activity measured in control treatments (empty nest and the nest with untouched eggs), and with three variables (the number of vertical trails in the trunk, the ant activity in all the vertical trails in 100 cm of the trunk, and the ant activity in the trail with the highest activity) was significantly higher in invaded areas than in uninvaded (Table 6a). There were no differences between pines and oaks (Table 6a). Time since the start of the experiment was nearly significant in only one variable, increasing trail numbers with time (Table 6a). Temperature was also nearly significant in the same variable, with fewer trails at high temperature (Table 6a). Finally, the size of the tree did not affect the normal ant activity in the tree (Table 6a).

Relative abundance of ants in the artificial nest was significantly different among treatments, among invasion status but not among tree types (Table 6b). The relative abundance of ants nearly decreased with higher temperatures (Table 6b) and ants visiting artificial nests were more abundant as the tree was bigger (Table 6b). Ants visiting artificial nests were more abundant in invaded than in uninvaded areas. Ants were significantly more abundant in the treatments with bird remains constituted by pierced eggs (pier. eggs) and feces and pierced eggs than when only feces were present, and significantly less abundant in both control treatments, the empty nest and the untouched eggs (Table 6b). The interactions of the treatment with tree type or with the invasion status were not significant (Table 6b). Finally, the interactions between the treatment and the invasion status were also significant (Table 6b), so that although relative ant abundance visiting the artificial nests was higher in invaded areas, the response of each ant species to the bird remains was different (Fig. 27a).

Table 6. Artificial nests. (a) Ant activity in invaded and uninvaded areas measured as the number of vertical trails in the trunk, the activity of all trails in 100 cm of the trunk, and the activity in the trail with highest activity. (b) Effects of the bird remain treatments on the ant activity in artificial nests in invaded and uninvaded areas. Significant effects are marked in bold.

a. Normal ant activity	**Number of trails** (N = 102)		**Ant activity (ants/min)** All trails (N = 96)		Highest trail (N = 101)	
	χ2	p	χ2	p	χ2	p
Invasion status	**6.58**	**0.010**	**9.55**	**0.002**	**10.04**	**0.001**
Tree type	0.95	0.330	2.79	0.095	1.00	0.318
Time since start experiment	5.64	0.059	1.66	0.435	2.91	0.233
Temperature	3.69	0.055	0.11	0.745	0.48	0.489
Trunk perimeter (tree size)	0.24	0.621	0.30	0.583	0.14	0.704

b. Activity in artificial nests	**Ant abundance** (N = 300)		**Post-hoc comparisons** 1. Treatment	
	χ2	p	Categories	p
1. Treatment	**16.27**	**0.003**	Empty nest vs entire eggs	0.551
2. Invasion status	**10.52**	**0.001**	Feces vs empty nest	**0.030**
3. Tree	3.23	0.072	Feces vs entire eggs	**0.019**
1*2	**15.68**	**0.003**	Feces vs pier. eggs	**<0.001**
1*3	4.20	0.380	Feces vs pier. eggs+feces	**0.002**
2*3	0.36	0.550	Pier. eggs vs pier. eggs+feces	0.959
Temperature	3.60	0.058		
Trunk perimeter (tree size)	**4.58**	**0.032**		

c. Ant recruitment to artificial nests	(N = 100)	
	χ2	p
1. Treatment	**159.75**	**< 0.001**
2. Invasion status	**71.50**	**< 0.001**
Tree type	0.09	0.769
Temperature	**10.39**	**0.001**
Trunk perimeter (tree size)	**13.94**	**< 0.001**

Native ants only recruited to the treatments containing pierced eggs, while the Argentine ant also recruited when only feces were present (Fig. 27b). There was no recruitment to untouched eggs or to empty nests, both considered as control treatments. Thus, we observed significant differences in the recruitment rate among the treatment levels and the invasion status (Table 6c). The type of tree did not affect the recruitment rate (Table 6c). Recruitment rate decreased with higher temperatures and was higher as the tree was bigger (Table 6c).

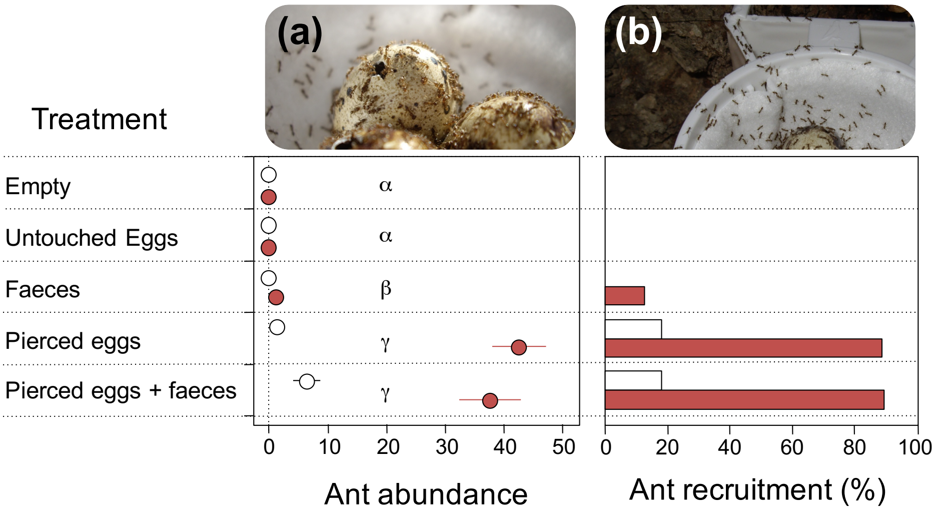


Fig. 27. Ants visiting artificial nests with different treatments of bird remains in invaded (red) and uninvaded (white) sites. The Argentine ant is the only ant visiting the artificial nests in invaded sites. (a) Relative abundance of ants in the nest. Abundance was standardized by the maximum ant abundance (350 and 190 ants in invaded and uninvaded sites, respectively). (b) Ant recruitment to the nest (*Photo credits: Paloma Alvarez-Blanco*).

Breeding performance

We compiled data on a total of 355 nest boxes, 169 located in invaded trees and 186 in uninvaded trees over five years of study (2013 – 2017). Great tits occupied one third of the available nest boxes in average of the five years of study (33% ± 2, mean ± SE, n = 5, Fig. 28) and significantly less nest boxes in the invaded sites compared to the uninvaded ones (LRT = 7.24, p = 0.007, n = 355, Table 7, Fig. 28). Breeding season started from the 10th to the 17th of March along the five years (13.8 ± 1.2, mean ± SE, n = 5) with no differences in the laying date of each nest between invaded and uninvaded sites (LRT = 0.09, p = 0.762, n = 113, Table 7). No differences among none of the other breeding variables were found between invaded and uninvaded sites, no matter if absolute variables: success, clutch size, brood size, number of fledglings, and nest weight; or relative variables: hatching success, fledgling success, and breeding success (Table 7). Results did not change when excluding the first year of observations (Table 7).

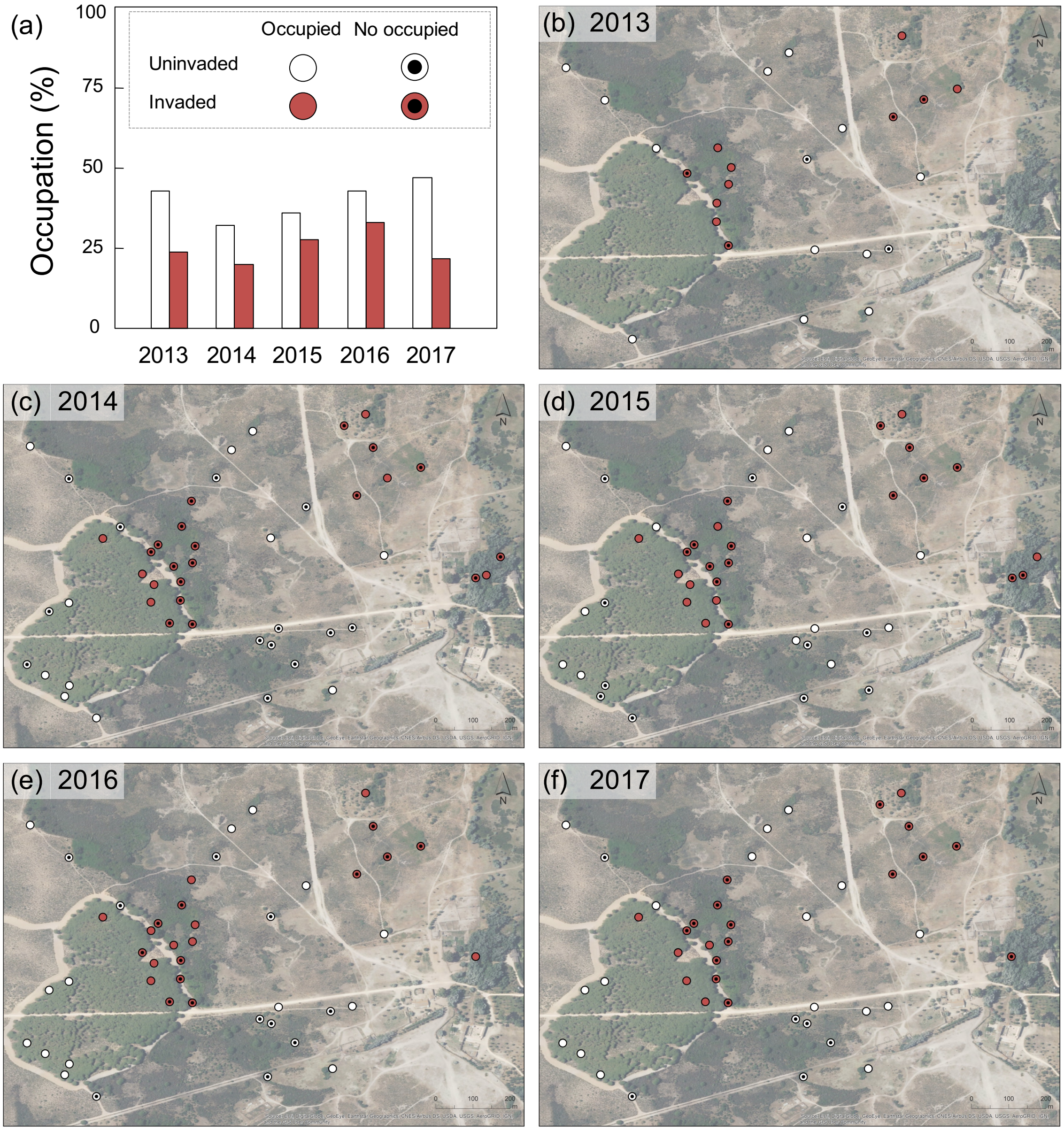


Fig. 28. Great tit occupation in invaded (red) and uninvaded (white) sites over the five years of monitoring (2013 – 2017). (a) Relative nest box occupation. (b-f) Trees with at least one nest box occupied (filled circles) or with no occupation (circles with a back dot). Each tree had three nest boxes in 2013 and one or two nest boxes in 2014 – 2017.

Table 7. Breeding variables measured in invaded and uninvaded areas (2013 – 2017). Nest box occupation (occupation) and success (success) are shown as percentages, and the other variables are average values of the nest (Mean ± SE). Sample size is referred to the number of nests and showed into brackets. Values are shown by year in invaded and uninvaded sites (Inv column, *I* or *U*, respectively). Significance test of the invasion status are shown for both 2013-2017, and 2014-2017 periods: *LRT*, p-value (*p*), and sample size (*n*). Nest weight was only measured in 2014 and F-value (*F*) is shown instead. Significant differences are marked in bold. See Box 2 for a description of each variable.

Year	Inv	Occupation (%)	Success (%)	Laying date	Clutch size	Brood size	Fledgling size	Hatching success	Fledgling success	Breeding success	Nest weight* (g)
2013	*I*	24 (33)	50 (6)	40 ± 8 (6)	7.2 ± 0.9 (5)	5.5 ± 1.5 (2)	4 (1)	62.5 ± 31.5 (3)	50 ± 50 (2)	33.3 ± 33.3 (3)	
	U	43 (42)	36 (14)	44 ± 5 (18)	8.4 ± 0.3 (8)	7.6 ± 0.5 (7)	6.8 ± 0.6 (5)	86.1 ± 6.7 (5)	64.3 ± 17.2 (7)	42.2 ± 18.1 (5)	
2014	*I*	20 (35)	67 (6)	36 ± 9 (7)	7.7 ± 0.6 (7)	6.9 ± 0.4 (7)	6.8 ± 0.9 (4)	90.1 ± 2.9 (7)	63.3 ± 20.3 (6)	55.9 ± 17.8 (6)	29.2 ± 4.2 (4)
	U	32 (37)	64 (11)	42 ± 7 (12)	8.8 ± 0.4 (12)	7.5 ± 0.5 (10)	7.1 ± 0.5 (7)	75.0 ± 8.5 (11)	65.4 ± 14.5 (10)	51.5 ± 12.6 (11)	26.7 ± 2.3 (7)
2015	*I*	28 (36)	100 (8)	44 ± 7 (8)	8.1 ± 0.4 (8)	7.3 ± 0.4 (7)	7.3 ± 0.4 (6)	91.9 ± 4.4 (7)	100 (6)	90.5 ± 4.9 (6)	
	U	36 (36)	85 (13)	36 ± 5 (13)	8.3 ± 0.3 (13)	8.1 ± 0.3 (13)	8.1 ± 0.3 (11)	97.2 ± 1.5 (13)	84.6 ± 10.4 (13)	82.8 ± 10.3 (13)	
2016	*I*	33 (33)	82 (11)	38 ± 4 (11)	8.3 ± 0.3 (10)	7.0 ± 0.5 (8)	6.8 ± 0.5 (8)	75.0 ± 10.8 (9)	96.9 ± 3.1 (8)	65.0 ± 11.7 (10)	
	U	43 (35)	80 (15)	42 ± 5 (15)	8.5 ± 0.4 (15)	6.5 ± 0.5 (15)	5.9 ± 0.6 (12)	76.4 ± 5.6 (15)	77.0 ± 10.7 (15)	57.1 ± 9.0 (15)	
2017	*I*	22 (32)	67 (6)	28 ± 3 (6)	9.2 ± 0.6 (5)	8.3 ± 0.3 (3)	8.5 ± 0.5 (2)	87.3 ± 6.4 (3)	100 (2)	90.9 ± 9.1 (2)	
	U	47 (36)	79 (14)	44 ± 6 (17)	8.1 ± 0.3 (15)	6.6 ± 0.6 (12)	6.7 ± 0.6 (11)	75.1 ± 8.7 (13)	90.5 ± 8.3 (12)	69.7 ± 10.3 (13)	
		LRT *p* *n*	*LRT* *p* *n*	*LRT* *p* *n*	*LRT* *p* *n*	*LRT* *p* *n*	*LRT* *p* *n*	*LRT* *p* *n*	*LRT* *p* *n*	*LRT* *p* *n*	*F** *p** *n**
2013 - 2017		**7.24 0.007 355**	0.01 0.926 104	0.09 0.762 113	0.35 0.556 98	0.09 0.770 84	0.01 0.941 67	0.26 0.608 86	0.84 0.359 81	0.0002 0.989 84	
2014 - 2017		**4.29 0.038 280**	0.09 0.767 84	0.02 0.882 89	0.10 0.750 85	0.8E-04 0.993 75	0.01 0.934 61	0.48 0.488 78	2.57 0.109 72	0.66 0.416 76	0.50 0.04 11

* Only data 2014

Individuals' parameters

Biometric variables (mass, tarsus and wing length) were all positively correlated among each other and negatively correlated to TRI. Besides, mass and wing length were positively correlated to CAT, mass was negatively correlated to GR, and wing length positively to GPX. UA was negatively correlated to TRI, CHOL, and TP. CHOL was positively correlated to TRI, TP and TBARS, and TRI and TBARS were also positively correlated to each other. GPX was positively correlated with CAT and negatively to TRI and SOD. Finally, GR was positively correlated with SOD (Table 8a). The principal components extracted from the multivariate analyses showed very low eigenvalues and first two factors only explained 48% of the variance (Table 8b). Results on the univariate models are thus, described below for fledglings and adults separately.

Table 8. Correlation among biometric and blood parameters of birds captured. (a) Correlation matrix with corresponding p-values. Significant correlations are marked in bold. (b) Scores for each variable and cumulative proportion of explained variance (eigenvalues) for the first two factors of a Principal Component Analyses. See Section 4.2 for a description of each variable. Data correspond to all individuals captured having data on all variables (n = 52).

a.	Mass	Tarsus	Wing	TRI	CHOL	TP	UA	TEAC	TBARS	CAT	SOD	GR	GPX
Mass		0.64	0.45	-0.28	-0.21	-0.02	0.16	0.00	-0.06	0.37	-0.22	-0.31	0.23
Tarsus	**p < .001**		0.47	-0.38	-0.21	-0.10	0.20	0.12	0.03	0.15	0.05	-0.26	0.14
Wing	**p = .001**	**p = .001**		-0.51	0.00	0.14	0.05	-0.13	-0.01	0.53	-0.25	-0.21	0.79
TRI	**p = .044**	**p = .006**	**p < .001**		0.59	0.19	-0.34	-0.18	0.40	-0.40	0.17	0.12	-0.34
CHOL	p = .136	p = .126	p = .982	**p < .001**		0.64	-0.35	-0.28	0.34	-0.20	-0.13	0.01	0.13
TP	p = .894	p = .495	p = .312	p = .186	**p < .001**		-0.34	-0.30	0.19	-0.01	-0.21	-0.03	0.20
UA	p = .249	p = .166	p = .703	**p = .014**	**p = .010**	**p = .013**		0.86	-0.09	0.11	-0.21	-0.15	-0.04
TEAC	p = .975	p = .393	p = .362	p = .208	**p = .041**	**p = .034**	**p < .001**		-0.01	0.01	-0.03	-0.12	-0.16
TBARS	p = .659	p = .814	p = .949	**p = .003**	**p = .014**	p = .171	p = .513	p = .931		0.07	0.22	0.05	-0.06
CAT	**p = .007**	p = .304	**p < .001**	**p = .003**	p = .154	p = .968	p = .437	p = .923	p = .644		-0.21	-0.12	0.56
SOD	p = .115	p = .708	p = .075	p = .216	p = .341	p = .143	p = .145	p = .824	p = .115	p = .133		0.36	-0.31
GR	**p = .027**	p = .068	p = .130	p = .380	p = .937	p = .832	p = .285	p = .391	p = .751	p = .380	**p = .009**		-0.09
GPX	p = .103	p = .324	**p < .001**	**p = .013**	p = .364	p = .163	p = .763	p = .255	p = .661	**p < .001**	**p = .027**	p = .525	

b.	Mass	Tarsus	Wing	TRI	CHOL	TP	UA	TEAC	TBARS	CAT	SOD	GR	GPX	Eigenvalue	Cumulative Proportion
FACTOR 1	0,93	0,85	1,04	-1,09	-0,66	-0,27	0,65	0,38	-0,37	0,89	-0,52	-0,54	0,79	3,56	0,27
FACTOR 2	-0,21	0,01	-0,71	-0,24	-0,92	-0,99	0,87	0,95	-0,34	-0,39	0,37	0,12	-0,83	2,63	0,48

Fledglings

In 2014, there were 11 successful nest boxes, 4 in invaded and 7 in uninvaded sites. One in the uninvaded sites was in a restricted area, so we got data on 10 nest boxes. From the 12 variables analyzed, we found significant differences among the invasion status in half of them (Fig. 29, Table 9). Chicks' mass and ratio wing/tarsus length (squared root-transformed) were significantly lower in invaded than uninvaded sites for all selected models (LRT =13.98, p < 0.001 and LRT = 10.08, p = 0.002 for mass and LRT = 9.53, p = 0.002 and LRT = 5.07, p = 0.024 for wing/tarsus ratio, n =69, Table 9). Triglycerides (log-transformed) were significantly lower in invaded than uninvaded sites in three out of four selected models (LRT = 5.24, p = 0.022; LRT=7.59, p = 0.006; and LRT = 4.71, p = 0.03, n = 61, Table 9). Total proteins were significantly lower in invaded than uninvaded sites in two out of four selected models (LRT = 8.20, p= 0.004 and LRT = 6,86, p = 0.009, n = 59, Table 9). TBARS (log-transformed) were significantly lower in invaded than uninvaded sites in one out of three selected models (LRT = 4.87, p = 0.027, n = 62, Table 9). GPX (squared root-transformed) was significantly higher in invaded than uninvaded sites in two out of four selected models (LRT = 6.07, p = 0.014 and LRT = 6.44, p = 0.011, n = 65, Table 9). No direct relation to the invasion status was detected for the other variables: CHOL, TEAC, UA, CAT, SOD, and GR (Table 9).

Then, focusing on the selected model with the lowest AIC value, chicks from invaded areas presented lower mass, wing/tarsus ratio, triglycerides, total proteins, and TBARS than those reared in uninvaded areas (Fig. 29, Table 9).

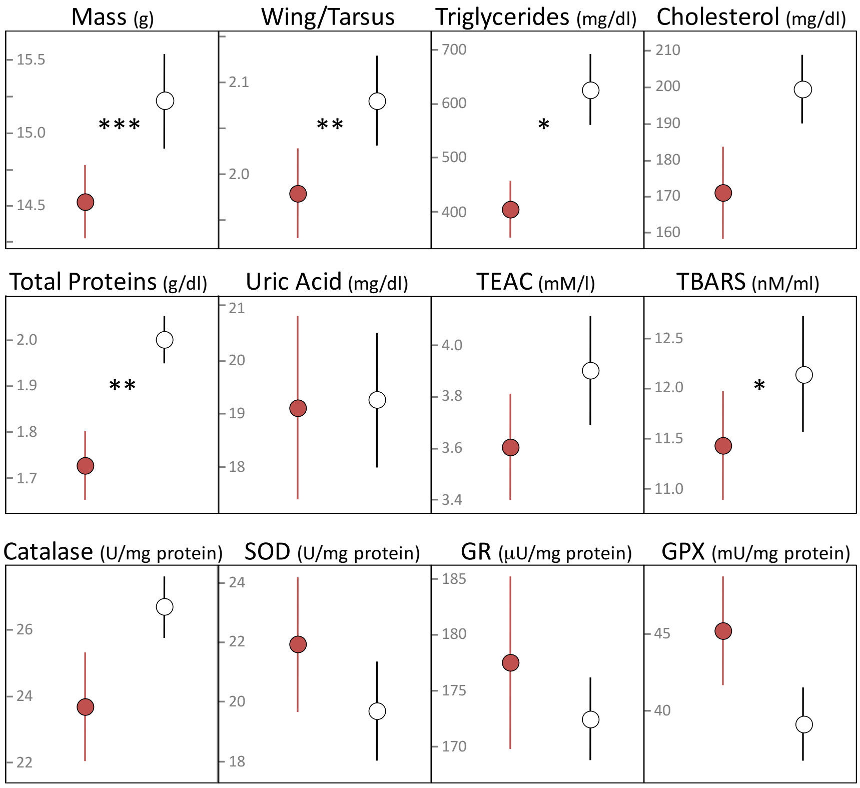


Fig. 29. Fledgling variables of biometry, biochemistry, and oxidative stress in invaded (red) and uninvaded (white) sites (mean ± SE). Significant differences in the model with the lowest AIC value are marked with asterisks (***p<0.001, **p<0.01, *p<0.05, see Table 9).

Table 9. Significant models for fledglings in 2014 taking into account biometry, biochemistry, and oxidative stress variables. Significance test of the invasion status (LRT, p-value) are marked in bold, and their trend direction is showed as positive (+) when values are higher in invaded areas or negative (-) when values are lower in invaded areas. Linear mixed models were selected by AIC (ΔAIC ≤ 2). All models included the nest box as random factor. Invasion status was considered as fixed factor in all models except in null models. Explanatory variables are: nestling age (age), fledgling size (FS), laying date (LD), longitude (x), and latitude (y). See Statistical Analyses section in the main text for geographical coordinates transformation.

RESPONSE VARIABLES	Model AIC	Explanatory variables	Invasion status		
			LRT	p-value	(+,-)
Mass	255.35	age FS LD x y	13.98	**<0.001**	(-)
	256.69	age FS LD	10.08	**0.002**	(-)
Wing/Tarsus (square)	151.20	age FS LD	9.53	**0.002**	(-)
	153.19	age FS LD x y	5.07	**0.024**	(-)
Triglycerides (log)	91.44	age LD x y	5.24	**0.022**	(-)
	91.55	age LD	2.23	0.136	-
	93.34	age FS x y	7.59	**0.006**	(-)
	93.44	age FS LD x y	4.71	**0.030**	(-)
Cholesterol (log)	11.14	age x y	1.10	0.295	-
	11.17	age FS	3.22	0.073	(-)
	11.48	age FS x y	1.27	0.261	-
	11.83	age LD x y	0.52	0.469	-
	11.92	age LD	1.08	0.298	-
	13.08	age FS LD	1.89	0.169	-
Total proteins	32.73	age FS	8.20	**0.004**	(-)
	33.88	age x y	1.35	0.245	-
	33.92	age FS x y	1.67	0.196	-
	34.63	age FS LD	6.86	**0.009**	(-)
Uric Acid	380.63	null model	-	-	-
	382.27	age LD	0.16	0.686	-
TEAC (log)	8.80	null model	-	-	-
TBARS (log)	8.62	age FS x y	4.87	**0.027**	(-)
	9.06	null model	-	-	-
	9.07	age FS LD x y	1.71	0.191	-
Catalase	433.55	null model	-	-	-
	434.61	age	1.54	0.215	-
	435.29	age x y	0.69	0.407	-
SOD (sqrt)	205.82	null model	-	-	-
GR (log)	-49.05	null model	-	-	-
GPX (sqrt)	212.94	null model	-	-	-
	213.56	age x y	6.07	**0.014**	(+)
	214.40	age	1.29	0.256	-
	214.92	age FS x y	6.44	**0.011**	(+)

Adults

We obtained data on 64% of the females and 54% of the males that bred with success in our study area in 2014. Due to the low occupation rate this is translated into a low sample size. We found no differences between adults breeding in invaded and uninvaded sites in any of the variables studied: mass, tarsus and wing length, TRG, CHOL, TP, UA, TEAC, TBARS, CAT, SOD, GR, and GPX (see Table 10 for mean values and test significance).

Table 10. Statistical models for adults breeding in 2014 taking into account biometry, biochemistry and oxidative stress variables. Significance test of the invasion status (F-value, p-value) by sex (female, male), and average values (mean ± se (n)) by invasion status (invaded, uninvaded). See Section 4.2 for a description of each variable.

RESPONSE VARIABLES		**FEMALE**				**MALE**			
		Invaded	*Uninvaded*	*F -test*		*Invaded*	*Uninvaded*	*F -test*	
Variable name	*Units*	Mean ± se (n)	Mean ± se (n)	F	p	Mean ± se (n)	Mean ± se (n)	F	p
Mass	g	16.6 ± 0.9 (3)	17.1 ± 0.3 (4)	0.25	0.637	16.5 ± 0.5 (3)	16.2 ± 0.2 (3)	0.46	0.535
Wing length	mm	69 ± 2 (3)	68 (4)	1.00	0.363	71 ± 1 (3)	74 ± 1 (3)	4.00	0.116
Tarsus length	mm	19.23 ± 0.43 (3)	19.17 ± 0.19 (4)	0.02	0.890	20.01 ± 0.20 (3)	19.36 ± 0.41 (3)	2.07	0.224
Triglycerides	mg/dl	136.68 ± 29.22 (3)	170.25 ± 38.56 (4)	0.42	0.545	140.21 ± 7.76 (3)	150.11 ± 27.47 (2)	0.19	0.693
Cholesterol	mg/dl	188.76 ± 24.06 (3)	204.52 ± 12.37 (4)	0.40	0.555	203.77 ± 8.46 (3)	227.55 ± 14.09 (2)	2.46	0.215
Total proteins	g/dl	2.25 ± 0.24 (3)	2.27 ± 0.09 (4)	0.01	0.944	1.90 ± 0.21 (3)	2.26 ± 0.38 (2)	0.83	0.430
Uric acid	mg/dl	15.81 ± 3.76 (3)	24.02 ± 4.21 (4)	1.94	0.222	21.47 ± 3.58 (3)	17.11 ± 3.45 (2)	0.68	0.470
TEAC	mM/l	3.20 ± 0.93 (3)	3.45 ± 0.59 (3)	0.06	0.827	3.31 ± 0.57 (3)	3.05 ± 0.03 (2)	0.12	0.749
TBARS	nmol MDA/ml	11.62 ± 0.68 (3)	12.42 ± 0.37 (4)	1.25	0.315	11.31 ± 1.27 (3)	17.07 ± 2.50 (3)	4.21	0.109
Catalase	U/mg prot	35.05 ± 5.45 (3)	39.70 ± 2.85 (4)	0.67	0.450	36.53 ± 2.24 (3)	32.21 ± 1.80 (3)	2.26	0.207
SOD	U/mg prot	10.84 ± 5.75 (3)	10.25 ± 1.99 (4)	0.01	0.916	13.48 ± 1.78 (3)	20.54 ± 9.11 (3)	0.58	0.489
GR	µU/mg prot	140.00 ± 15.28 (3)	155.00 ± 8.66 (4)	0.84	0.402	170.00 ± 5.77 (3)	136.67 ± 12.02 (3)	6.25	0.067
GPX	mU/mg prot	17.62 ± 18.09 (3)	124.46 ± 15.05 (4)	0.09	0.781	123.85 ± 20.53 (3)	77.70 ± 17.22 (3)	2.97	0.160

Discussion

Great tits did not show differences in the breeding performance between sites invaded and uninvaded by Argentine ants. However, significantly more nest boxes were occupied in uninvaded areas. Besides, nestlings reared in invaded sites exhibited an impoverished condition compared to those reared in uninvaded sites, indicated by lower mass and size, and altered physiological parameters related to both their nutritional state and their oxidative balance. Although other studies have examined the effects of the Argentine ant invasion on bird reproductive performance (Estany-Tigerström *et al.* 2013; Krushelnycky *et al.* 2001), little is known about the physiological consequences of breeding in Argentine ant invaded land. Here, we found negative effects of breeding in areas invaded by Argentine ants that hamper development on early stages and likely have lagged consequences.

Great tits in our study area occupied less frequently boxes placed in trees invaded by the Argentine ant than those with native ants. Great tits, like other insectivorous birds, chose their breeding site mainly by habitat structural cues of resource availability (i.e., “structural-cues hypothesis” Marshall and Cooper 2004; Smith and Shugart 1987). Estany-Tigerström *et al.* (2013) did not find differences in the occupation rates of the blue tit (*Cyanistes caeruleus*) between plots invaded and uninvaded by the Argentine ant; they suggested that invaded areas are ecological traps for insectivorous birds because they suffer from prey depletion caused by the Argentine ant, but habitat structural cues the birds rely on to chose nesting site remain similar in invaded than in uninvaded areas (Estany-Tigerström *et al.* 2013; Pons *et al.* 2010). Our experimental design allowed great tits to forage in both, invaded and uninvaded areas, as the distance between boxes from invaded and uninvaded areas was small, in some cases just a few meters. Parent great tits can travel much longer distances to obtain food for their chicks (Naef-Daenzer 1994), so the hypothesis that the Argentine ant influences negatively the surrounding areas of the nest, impinging on the food resource availability is not supported in our study system.

Birds also select breeding sites regarding the microhabitat. For instances, the selection of certain nest orientation is known to provide suitable microclimatic conditions for reproduction (Ardia *et al.* 2006; Goodenough *et al.* 2008a; Goodenough *et al.* 2008b) and the preference for higher nesting sites has been suggested as an adaptive behavior to avoid predation (Forstmeier and Weiss 2004; Nilsson 1984). Here, we found great tits seemed to avoid breeding in nests placed in invaded sites, which might be the consequence of previous negative encounters along the last decades since invasion. Interference competition among birds and ants has been suggested to change bird behavior, avoiding foraging in trees with ant activity (Aho *et al.* 1997; Haemig 1996; Philpott *et al.* 2005). Haemig (1996) suggested great tits evolved behaviors to reduce exposure to ant toxins based on observations of ant aggressions to the birds. For example, the anting behavior could have the dietary function of get rid of the noxious substances prior ingestion rather than a fumigatory function (i.e., the "ant toxin avoidance hypothesis", Eisner and Aneshansley 2008; Judson and Bennett 1992).

The Argentine ant is known by its aggressiveness and some studies have attributed bird nesting failure to the attacks of this invasive ant (Table 1). Unlike other invasive ants. (i.e., *Solenopsis invicta, S. xyloni*, Hooper-Bui *et al.* 2004; Seymour 2007), the Argentine ant has never been reported to break the egg's shell (see Hooper-Bui *et al.* 2004; Suarez *et al.* 2005), in line with our results. Some of the above-mentioned studies attributed nest failure to the invasive ant if they observed nests infested by ants and feeding on chicks' corpses (Mejías *et al.* 2017; Sockman 1997; Suarez *et al.* 2005) while most of the studies referred the observation of a unique nest where the Argentine ant was suggested to be responsible for the chicks' death (Delibes 2005; Flores *et al.* 2017; Hooper-Bui *et al.* 2004; Peterson *et al.* 2004). Recently, Flores *et al.* (2017) reported observations of several disturbed adults covered with Argentine ants, a single chick was found agonizing and partially predated by ants, which were found in the necropsy in rectum and nostrils. We found Argentine ants were more abundant and recruited more frequently than native ants in pierced eggs in artificial nest (Fig. 27). Suarez *et al.* (2005) presented similar results when studying the effect of the Argentine ant on ground-nesting birds and recently, Varela *et al.* (2018) showed how quickly Argentine ants overwhelmed a simulated pierced egg (75% of probability to be attacked after 5min of exposure). Boieiro *et al.* (2018) graphically showed two cases where the invasive ants were recruiting in large numbers and fed on the egg contents while the nestlings were hatching. Although both, native and invasive ants were attracted to the pierced eggs in our artificial nest experiment, the Argentine ant was the most abundant and recruited more frequently to the resource. Throughout the breeding season we observed ants, both invasive and native, feeding on chick corpses (see native

Box 4. Interactions between ants and chicks observed during monitoring

Native ants, such as *Lasius grandis* (left) and *Crematogaster scutellaris* (right), were observed feeding on chicks' corpses.

The native ant *Crematogaster scutellaris* was also observed feeding on eggs.

The invasive Argentine ant (*Linepithema humile*) was observed feeding on chick's corpses, but also attacking alive chicks.

Argentine ants were observed biting two chicks approximately two days old.

A female breeding in an invasive site was observed to have an uncommon behaviour. It was found huddled under two seven-days-old chicks in a messy nest full of **Argentine ants** coming from an active trail in the trunk. Those chicks were found dead in the following revision, three days later, one was already dry and the other recently death.

ants in Box 4). However, only in two situations the Argentine ant was found to disturb live chicks and the nests failed, while we did not observe any similar case with native ants (Box 4). Lambrechts *et al.* (2008) did reported observations of the native ant, *Crematogaster scutellaris*, (which is the main native ant in the uninvaded areas) disturbing live chicks or predating on death chicks of passerine species.

Despite attributions of nest failure to the Argentine ant (Table 1), it is generally considered that the Argentine ant has a low impact in bird reproductive success (see reviews in Lach and Hooper-Bui 2010; Suarez *et al.* 2005; Table 1). In agreement with previous studies (Estany-Tigerström *et al.* 2013; Krushelnycky *et al.* 2001), we did not find any difference in the breeding success between sites invaded and uninvaded by the Argentine ant. To explain the lack of differences, Krushelnycky *et al.* (2001) suggested that the low temperatures in the nest of burrowing birds could limit the Argentine ant abundance; while Estany-Tigerström *et al.* (2013) suggested that parental extra effort might compensate the loss of food resources due to the Argentine ant invasion. However, none of the above explanations are likely to apply here because first, temperature in the great tit nests is thought to be a thermal gain benefit for ants rather than a constraint (Mitrus *et al.* 2016); and second, food resource is scarce but equally available in our invaded and uninvaded sites. Therefore, one could think the Argentine ant is not affecting great tits reproduction in our study area.

However, we did find differences in the physiological status and growth of the progeny. Great tits nesting in invaded sites reared poorer quality offspring. Fledglings in invaded areas were lighter and exhibited lower wing:tarsus ratio. Although Estany-Tigerström *et al.* (2013) did not find any relation between the Argentine ant invasion and blue tit nestling's conditions (body mass, tarsus length, and carotenoid-based plumage coloration), birds nesting in areas infested by other invasive ants such as the red imported fire ant and the invasive European fire ants (*Myrmica rubra*) were adversely associated to chick growth rates or body condition (DeFisher and Bonter 2013; Ligon *et al.* 2012). RIFA may impinge directly chicks by stinging and/or altering their behavioral pattern (Giuliano *et al.* 1996; Pedersen *et al.* 1996) or indirectly by increasing the foraging distances of their parents (Ligon *et al.* 2012). The European fire ants also increase the erratic breeding behavior of adults, which may hinder embryonic development (DeFisher and Bonter 2013). Effects on the offspring have been also reported with the native ants *Formica rufa*-group, which abundance was adversely associated to body mass, tarsus length, and subcutaneous fat index of treecreeper offspring (*Certhia familiaris*) (Jäntti *et al.* 2007). Our results seem to be in line with these previous studies, and the Argentine ants may also affect negatively chicks by disturbance. Although the Argentine ant does not have a functional stinger such as the fire ants or formic acid arsenal such as Formicinae ants, it is known to be aggressive and more abundant than native ants in the invaded trees (Table 6, Fig. 27). Besides, it has a powerful venom that has recently been shown to kill amphibians (Section 2), and its effect on bird has not been tested yet. Therefore, its abundance, aggressiveness, and venom may hamper chicks by direct disturbance.

Moreover, we found nutritional parameters to be reduced in chicks grown in invaded sites. Lipids constitute one of the primary physiological fuel used in starvation (McCue 2010) and proteins have functional or structural role. Although oxidative balance is very complex and interpretation of isolated parameters should be taken with cautious, our results indicate it is altered in invaded areas. Conditions during early development can have long-term consequences in the adulthood (Costantini 2014; Lindström 1999; Monaghan 2008). For example, it has been showed how poor condition of chicks diminishes post-fledging survival (Naef‐Daenzer *et al.* 2001; Rodríguez *et al.* 2016), fecundity (Clarke 1995) and blood parasite resistance (Stjernman *et al.* 2008). Unlike genetic factors, environmental factors operating during early development can affect the whole cohort simultaneously (Lindström 1999), causing negative effects at the population level.

Parents might be able to compensate any potentially negative effect nestlings could suffer from invasive ants by increasing their foraging effort (Estany-Tigerström *et al.* 2013; Ligon *et al.* 2012). Foraging areas in these studies differ between invaded and uninvaded areas. Although we cannot obviate the role parents play on offspring quality, including both, gens and parental care, in our study site invaded and uninvaded areas shared foraging areas. In agreement with that, we did not find differences in adults breeding in invaded or uninvaded sites in the body size or physiological parameters analyzed (Table 10), although we should be cautious due to the low sample size.

Here, we found a passerine bird was negatively affected when breeding on areas invaded by Argentine ants. It is probable that the suboptimal conditions experienced at the southern margin of the distribution have allowed detecting the impacts of the Argentine ant. Thus, our results suggest that the subtle impacts of invasive species might act in synergy with other global change drivers, such as climate.

General Discussion

Invasive species are necessarily obligated to interact with native species of the recipient ecosystem, regardless of whether the consequences are direct or indirect, positive or negative, more obvious or subtle. This thesis focuses on the often overlooked indirect and subtle effects of invasive species on ecosystems by exploring the interactions of the invasive Argentine ant with native vertebrates. My results show that the Argentine ant invasion exerts ecological impacts on vertebrates through indirect and previously unsuspected means which vary in magnitude. Native vertebrates in early stages of development showed reduced survival, growth, and altered physiological parameters when they developed in the presence of Argentine ants and/or in invaded areas. Vertebrate adults also modified their habitat use (i.e. for breeding or foraging) in invaded areas, although responses differed among species. In addition, native predators shifted to non-ant prey in invaded areas, probably due to the displacement of suitable ant prey in these areas. Surprisingly, however, the Argentine ant was found to prey upon newly emerged amphibians, changing its role from prey to predator. The unexpected lethality of its venom is described for the first time as the mechanism used to subdue vertebrate fauna. Furthermore, sublethal effects of the venom likely contribute to the previously mentioned indirect impacts. This thesis highlights the importance of taking into account the bottom-up effects, exploring the proximate effects, and describing the specific mechanisms responsible when assessing the effects of invasive species on native ecosystems.

Bottom-up effects: effects of the invasive prey on native predators

This thesis corroborates previous findings that the Argentine ant displaces the native ant community to become virtually the only ant species both on the ground and in the trees (Angulo *et al.* 2011; Carpintero *et al.* 2005). Section 1 of this thesis shows how native ants' displacement scales up to higher trophic levels: in the studied area of Doñana, the native amphibian community shifted its diet to non-ant preys in invaded areas. Using the existing theoretical framework that describes potential relationships between native predators and invasive preys, I examine some well-known hypotheses that largely focus on the success of the introduced prey, such as the enemy release hypothesis, the exotic prey naïveté hypothesis, the specialist-generalist hypothesis, or the evolution of increased competitive ability of the invasive prey (Callaway and Ridenour 2004; Carlsson *et al.* 2009; Catford *et al.* 2009; Ricciardi *et al.* 2013; Sax *et al.* 2007). By considering the effects on predators in each of these scenarios, I expand these hypotheses in order to understand how invasive prey affects native predators. My results show that native predators with greater dietary specialization are more likely to be negatively affected by prey displacement (Pintor and Byers 2015). Although the studied native amphibians were less ant-specialized than expected (as revealed by isotopic analyses), all diets included ants to some extent. The degree of ant-specialization of the native predator seemed to be negatively related to their abundance in invaded areas, but not in uninvaded areas. The most myrmecophagous species, *Epidalea calamita*, seemed to avoid invaded areas, while the species that was the least ant-specialized, *Pelobates cultripes*, showed the opposite trend. In this, my results were similar to those found for the coastal horned lizard, *Phrynosoma coronatum*, a highly specialized ant-predator whose populations are in decline in areas invaded by Argentine ants (Suarez and Case 2002; Suarez *et al.* 2000). However, unlike *P. coronatum*, *E. calamita* do consume Argentine ants and even increased their consumption in subsequent trials, although they demonstrated a preference for native ants. To date, with the exception of the coastal horned lizard, threats of Argentine ant invasion to other vertebrate predators have not been evaluated, even though other amphibian species are known to feed on Argentine ants in invaded areas (e.g., Ito *et al.* 2009). However, myrmecophagic invertebrates seem to benefit from the Argentine ant invasion, including pit-building antlions (Glenn and Holway 2008) and spiders (Takahashi *et al.* 2018; Touyama *et al.* 2008). Therefore, beyond the issue of dietary specialization we should also consider the manner in which native predators might use the novel prey as a resource, including their ability to detect, capture, and consume it (Glenn and Holway 2008; Monzó *et al.* 2013; Suarez and Case 2002; Suarez *et al.* 2000). Novel traits of invasive species (i.e. behavior, toxins) and their associated indirect costs (i.e. reduced survival and development) may also affect native species in a different manner, as discussed below.

Top-down effects: when the invasive prey becomes a predator

The Argentine ant is prey, but also may act as a predator of native vertebrates. Although previous studies have attributed avian nest failure to attacks of this invasive ant, all evidence was anecdotal (Table 1). In Section 2, I reveal for the first time in the literature the Argentine ant's mechanism of predation on newly metamorphosed amphibians. I show how the ant uses iridomyrmecin, its main defensive compound, to penetrate toadlets' skin to the inner tissues, causing lack of coordination, paralysis, and death. Similar signs have been reported from evaluations of the toxic effects of iridomyrmecin on several insects, including the Argentine ant (Hemp and Dettner 1997; Pavan 1952a; Welzel *et al.* 2018). Thus, in spite of the physiological differences between vertebrates and invertebrates, Argentine ant venom is effective on both taxa. The Argentine ant's role as a predator is not obvious, and this has likely contributed to the underestimation of its impact on vertebrates. Indeed, although the Argentine ant is omnivorous, it has been shown to display a strong preference for carbohydrates in introduced areas (Abril *et al.* 2007; Angulo *et al.* 2011; Newell and Barber 1913; Rowles and Silverman 2009; Tillberg *et al.* 2007). Furthermore, unlike the red imported fire ant (*Solenopsis invicta*), the Argentine ant lacks a functional stinger, ant its defensive compounds were not previously documented as toxic for vertebrates after testing in humans, dogs, and rodents (Pavan and Ronchetti 1955). Therefore, this thesis brings to light a mechanism of vertebrate predation by an invasive species that has been previously neglected.

This thesis demonstrates that the toxicity of the Argentine ant is dose-dependent when applied topically to the skin of juveniles of the three amphibian species tested: natterjack toadlets (*E. calamita*), western spadefoot toadlets (*Pelobates cultripes*), and Mediterranean tree froglets (*Hyla meridionalis*). Because of this, newly metamorphosed amphibians are expected to be more sensitive than juveniles, who are themselves more sensitive than adults. Similar dose-dependent responses have been found for the venom of other ants (e.g., red imported fire ants on endangered Houston toads, *Bufo houstonensis*, and on eastern fenced lizards, *Sceloporus undulatus*, Freed and Neitman 1988; Read *et al.* 1978; Robbins and Langkilde 2012, and for the venom of *Pachycondyla* ants on crickets, Orivel and Dejean 2001).

This thesis also demonstrates that the toxicity of Argentine ant venom is species-specific. This is very likely due to differences in physiology and anatomy (e.g., absorption, metabolism) among amphibians. In addition, my results suggest that the risk of envenomation (which encompasses more than just toxicity) likely differs among species depending on their behavior when confronted with an ant attack. Although I did not specifically address the defensive responses of the amphibian species, I observed different behaviors: the stripeless tree froglet, with long legs and large adhesive discs, jumped to escape Argentine ants; the natterjack toadlets buried themselves using their digging spades; and the western spadefoot toadlets excreted toxins from their parotoid glands. Such species-specific differences have also been described in previous publications. For example, vulnerability to the toxic invasive cane toad, *Rhinella marina*, has been shown to differ among native species (Cabrera-Guzmán *et al.* 2015; Crossland and Alford 1998; Shine 2010, 2014), and both morphological and behavioral traits play an important role in avoiding lethal attacks of the red imported fire ant (Langkilde 2009; Long *et al.* 2015). Therefore, these results reveal that lethal and sublethal effects of the Argentine ant invasion on young vertebrates are species-specific, which supports the idea that the effects of invasive species depend greatly on features of the recipient ecosystem (Catford *et al.* 2009; Kumschick *et al.* 2015; Pyšek *et al.* 2012; Ricciardi *et al.* 2013). Even with the species-specific and dose-dependent effects in mind, though, it seems that the toxicity of this venom is likely context-independent. That is, the venom seems to be toxic for most native terrestrial amphibians (as it is for arthropods). This means that we need much more research to assess not only the venom's toxicity but also the defensive responses of amphibian species in ecosystem worldwide that have been successfully invaded by the Argentine ant.

From direct to indirect effects and from individual to population level

One of the important results of this thesis is that invasive species cause sublethal effects on native species that can potentially carry delayed consequences. Reduced body condition was demonstrated in both amphibians (when raised on a diet supplemented by with Argentine ants; Section 3) and birds (avian offspring reared in invaded versus uninvaded sites; Section 4). Other invasive species that are well known for their toxicity, such as the red imported fire ant and the cane toad, also incur sublethal effects on native species (Allen *et al.* 1997; Langkilde and Freidenfelds 2010; Llewelyn *et al.* 2009; Long *et al.* 2015; Myers *et al.* 2014). It has been assumed that reduced development in the early stages of life decreases the individuals' fitness (Lindström 1999; Monaghan 2008), which might have consequences at a population level. Indeed, this thesis provides evidence for changes in habitat use in invaded areas by the terrestrial amphibian community (foraging, Section 1) and by the great tit population (reproduction, Section 4). Similar changes in habitat use have been previously described for the horned lizard, a recognized ant-specialist, and attributed to the effect of prey displacement by Argentine ants (Fisher *et al.* 2002). Moreover, other vertebrates that do not feed on ants, such as shrews and insectivorous birds, also change their habitat use in areas invaded by Argentine ants (Laakkonen *et al.* 2001; Pons *et al.* 2010). This thesis casts light on unexpected and subtle effects of the Argentine ant invasion on vertebrates, such as lethal and sublethal impacts on early developmental stages of different taxa, which might induce long-term effects that scale to the population and community level. Long-term studies are encouraged in order to fill the gaps between the effects found on young vertebrates and changes at population and community levels.

Iridomyrmecin: Argentine ant venom and proposed future lines of study

Venoms help to modulate predator-prey relationships by serving as mechanisms of defense and prey capture, and occasionally both functions simultaneously (i.e. *Solenopsis invicta*, Blum 1996; Pasteels *et al.* 1983; Schmidt 2009). Despite advances in microanalytical techniques in the last century and increased collaboration among chemists and ecologists, the ecological function of most arthropod venoms remains understudied. In the case of iridomyrmecin, I benefited from the entomological, chemical, and pharmaceutical research of Mario Pavan in the mid-twentieth century, who isolated this compound from the Argentine ant (formerly *Iridomyrmex humilis*), named it, and described it as an insecticide and antibacterial (Pavan 1950, 1951, 1952a,b,c,d; Pavan and Nascimbene 1948a,b; Pavan and Ronchetti 1955). Although iridomyrmecin in the Argentine ant has been viewed as an important volatile compound in trail and alarm pheromones, its potential for use as venom has been overlooked from Pavan’s work onwards, and has always been limited to insects and bacteria (review in Appendix 1). This thesis takes the novel step of demonstrating lethal and sublethal impacts of Argentine ant venom on invaded ecosystems.

The fact that the effect of this venom has been overlooked in such a successful invasive species open avenues for exploring several questions that, in my opinion, deserve further study. The following sections discuss the implications of the venom for other taxa, the venom’s effects in the ant’s native range, the role of the venom in the success of the Argentine ant, and the phylogenetic history of the venom.

Effects of venom on vertebrates in the invaded range

The toxicity of iridomyrmecin for vertebrates has only been previously tested on mammals, which showed very low or no toxicity even at high doses, suggesting it is innocuous to homeothermic species (i.e. rats, dogs, and humans, Pavan and Ronchetti 1955). Here, for the first time, I tested and verified its toxicity on amphibians when applied topically, but it remains unknown if this venom would act effectively against other vertebrate taxa.

As an example, if the venom affects reptiles in a similar manner, it may have played a role in the decline of the coast horned lizard in areas invaded by the Argentine ant (Suarez and Case 2002; Suarez *et al.* 2000). In fact, the results presented in this thesis bear similarity to those demonstrated on horned lizards: lower densities in invaded areas (Fisher *et al.* 2002, Section 1), lower growth and survival when feeding on the invasive ant (Suarez and Case 2002, Section 3), lower preference for the Argentine ant compared to native ants (Suarez *et al.* 2000, Section 1), and prey shift in invaded areas (Suarez *et al.* 2000, Section 1). Besides the small size and aggressive behavior of the Argentine ant, unpalatability has been suggested as the reason the ant is an unsuitable prey for the coast horned lizard (Suarez *et al.* 2000). Although this thesis failed to show toxicity of the Argentine ant via oral consumption, higher ant doses, simulating those potentially found in highly myrmecophagous predators such as horned lizards, might show oral toxicity. If external toxicity on horned lizards does occur, the Argentine ant might also cause sublethal or lethal effects that have not yet been detected. Unlike amphibians, reptiles have a stronger barrier to prevent external envenomation—their skin is not as permeable and their scales act as armor, hindering the absorption of the Argentine ant's venom. However, the red imported fire ant bypasses the external barrier of native fenced lizards by raising a scale and injecting venom into the underlying soft flesh (Langkilde *et al.* 2017). Non-stinging ants or those whose stinger is not functional are known to bite to bypass external defensive barriers (a predator's skin) and deploy the venom in the wound to enable absorption (Cavill and Clark 1971; Schmidt *et al.* 1989). Thus, it is possible that the Argentine ant bites and deploys its venom in a similar manner on lizards.

Might birds and mammals be vulnerable to Argentine ant venom? Some studies have pointed to the Argentine ant as the factor responsible for the death of chicks

(Table 1) and here I found that chicks were smaller in invaded sites, and with altered physiological parameters, compared to those reared in uninvaded sites (Section 4). Likewise, the abundance and distribution of Crawford's grey shrew (*Notiosorex crawfordi*) has been negatively correlated to the presence of Argentine ants (Laakkonen *et al.* 2001). Beyond this publication and those metioned in the previous paragraph, though, few studies have addressed the impacts of the Argentine ant on vertebrates and none have considered its toxicity (with the exception of Pavan and Ronchetti 1955). Although Pavan and Ronchetti (1955) reported only low toxicity of iridomyrmecin for mammals in terms of mortality, sublethal doses may still hamper the growth and development of mammals' offspring. In addition, Pavan and Ronchetti's results differed depending on the solvent used, but there is currently no information on whether there is an effect of the *real* biological solvent, the minor compounds that the ant deploy together with iridomyrmecin. Indeed, other pygidial compounds may also function as defensive products (e.g. dolichodial, Cavill *et al.* 1976; Welzel *et al.* 2018) or help the venom to reach and stay on the target (e.g. iridodials, Attygalle and Morgan 1984). Therefore, further research is needed before toxicity for homeothermic species can be discounted. Taking together, our results clearly demonstrate that iridomyrmecin is toxic for amphibians and suggest that it could also be toxic for other vertebrates.

Effects of venom on vertebrates in the native range

To the best of my knowledge, the interaction of the Argentine ant with vertebrates in its native range has not yet been assessed. The study of invasive species' relationships in their native ecosystems may help to elucidate the causes and consequences of their invasion (Hierro *et al.* 2005; Mack *et al.* 2000; Pedersen *et al.* 2006; Vogel *et al.* 2009). Particularly, it may clarify whether the lethal and sublethal impacts of the Argentine ant on amphibians that are described in this thesis are due to predator naïveté, a lack of coevolution with the invasive species, or both. Chemical defenses play an important role in predator-prey interactions (Berenbaum 1995; Jackson *et al.* 2016; Phillips and Shine 2007; Schmidt 2009; Uemura *et al.* 2017) and some invasive species may expand into native ecosystems with the aid of novel toxins (novel weapons hypothesis, e.g., ants Sih *et al.* 2010, amphibians Hagman *et al.* 2009, and plants Callaway and Ridenour 2004). Amphibians from the native range may have evolved defense mechanisms (behavioral, anatomical, and/or physiological) that allow them to coexist with and/or feed on the Argentine ant. For instance, some amphibians are known to inhabit ant nests without suffering damage, which has been attributed to chemical deterrents in their skin or substances that mimic those of the ants (Dejean and Amiet 1992; Rödel and Braun 1999; Schlüter and Regős 1996). For one amphibian (*Phrynomantis microps*), the adoption of submissive behavior while being examined by African ants (*Pachycondyla tarsatus*) is thought to help it to estivate in their nest (Rödel and Braun 1999). The evolution of a chemical arms race has also lead some species to develop mechanisms for the detoxification of ant venom, such as specialist predators (Schmidt *et al.* 1989; Sherbrooke and Schwenk 2008) or competitor ants (LeBrun *et al.* 2014).

In its native range, the Argentine ant colonies are much smaller than in the introduced range and, far from being almost the only ant species, *L. humile* is but one member in a larger ant community (Heller 2004; LeBrun *et al.* 2007; Pedersen *et al.* 2006; Tsutsui *et al.* 2000). In fact, it coexists with other dominant species such as *Solenopsis* sp. and *Pheidole* sp. (Fernández 2003; Heller 2004; Wilson 2003), which are also the main prey of myrmecophagous amphibians (Berazategui *et al.* 2007). Therefore, in its native range, the Argentine ant might not exert sufficient selective pressure on amphibians when compared to other native ant species. However, the relationships between the Argentine ant and amphibians in the native range have not yet been explored, and at least 73 amphibian species share their distribution range and microhabitats with populations of native Argentine ants (see Fig. 21 in Section 2). If amphibians in the native range show low susceptibility to iridomyrmecin, this would point to the existence of an adaptation (i.e. they have been subjected to strong selective pressure) and would suggest that a lack of coevolution between the invasive ant and amphibians from the invaded range is responsible for the latter group's vulnerability. Instead, if amphibians in the native range are similarly affected by the venom as those in the invasive range are, it would signify a lack of selective pressure from the Argentine ant's venom (at least at a physiological level). This would suggest that the impact of the venom in invaded areas could arise more from the colony structure of the Argentine ant than from the physiological consequences of the venom itself. In order to test these hypotheses, the following questions should be addressed: Is the Argentine ant less aggressive toward amphibians in its native range than in the invasive range? Is the

Argentine ant's venom similarly toxic to native amphibians in both ranges? Is there a species-specific response also in the native range?

Could the venom help to explain the success of the Argentine ant?

Throughout this thesis I have focused on the consequences of invasion. However, my findings may also be applied to elucidate the causes of the Argentine ant's success. The Argentine ant is the second most-studied invasive species in the world (Pyšek *et al.* 2008) and one of the main reasons for its success is thought to be the formation of large supercolonies in the introduced range (unicoloniality) (Helanterä *et al.* 2009; Holway *et al.* 2002; Holway *et al.* 1998; Pedersen *et al.* 2006; Suarez *et al.* 2008). In fact, though, other invasive ants have been documented or inferred to present unicoloniality (*Solenopsis invicta*, *Pheidole megacephala*, *Wasmannia auropunctata, Anoplolepis gracilipes, Lasius neglectus*, *and Nylanderia fulva*) (Eyer *et al.* 2018; Holway *et al.* 2002; Ugelvig *et al.* 2008). In spite of great efforts made to disentangle the basis of unicoloniality and the differential success of introduced supercolonies, the relationship remains unclear (Blight *et al.* 2012; Blight *et al.* 2017; Giraud *et al.* 2002; Helanterä *et al.* 2009; Pedersen *et al.* 2006; Suarez *et al.* 1999; Tsutsui *et al.* 2000; Vogel *et al.* 2010). Previous studies have focused on recognition approaches, involving genetics, chemical, and behavioral studies (Abril and Gómez 2011; Blight *et al.* 2012; Blight *et al.* 2017; Brandt *et al.* 2009; Giraud *et al.* 2002; Vogel *et al.* 2010), but the contribution of defensive compounds has not yet been explored in this context. Based on the potential significance of the Argentine ant's venom (Section 2), it is possible that iridomyrmecin may play a role in the success of the introduced supercolonies. Here, I propose two alternative predictions based on the venom optimization hypothesis (Morgenstern and King 2013; Wigger *et al.* 2002) and the evolution of increased competitive ability hypothesis (following e.g., Blossey and Notzold 1995; Catford *et al.* 2009; Müller-Schärer *et al.* 2004; Ricciardi *et al.* 2013). Iridomyrmecin might be used as a defensive and offensive tool to dominate introduced environments. If this is the case, it will be present in higher quantities in the most-successful colonies (venom optimization hypothesis). If instead it does not contribute to the success of colonies, it might no longer be necessary, and be present in lower quantities in the most-successful colonies. In the latter case these successful colonies might reduce the costs associated with venom production and reallocate the surplus energy and/or space to other resources that could contribute to their dominance (i.e. greater foraging efficiency, larger food reservoir; evolution of increased competitive ability hypothesis). Consistent with the first hypothesis, the red imported fire ant enlarges its poison gland and injects higher quantities of venom after colony flooding (Haight 2006; Papillion *et al.* 2011), although another study found that the ants failed to increase their aggressiveness after flooding (Huang *et al.* 2016). Likewise, the Argentine ant has been noted to increase the use of chemical interference competition when migrating, that is, during the time immediately preceding the invasion of new territory (Crowell 1968; Fluker and Beardsley 1970; Lieberburg *et al.* 1975). Therefore, the chemical arsenal of the Argentine ant may contribute to the success of introduced supercolonies and should be further explored.

Does the venom have a phylogenetic signal? Does it contribute to invasiveness in other species?

In the study of invasive ants, chemical defenses provide unique opportunities for research on ecology and behavior, as shown in this thesis (Section 1, Section 3). Such defenses are also fascinating from the perspective of evolutionary biology, as argued by Schmidt (2009): “insect venoms have played an enormous role in ecology and evolution”. Formic acid was the first venom described in ants and is apparently exclusive to and ubiquitous in the Formicidae subfamily (Hefetz and Blum 1978; Schmidt 1986). Mario Pavan selected the Argentine ant as a model to test, for the first time, the hypothesis that formic acid was not the only ant chemical with offensive and defensive functions (Pavan 1952d). Venoms of dolichoderine ants (i.e. iridoids, ketones) have been poorly studied compared to peptide- and protein-rich venoms (stinging ants), formic acid (formicine ants), or alkaloids (e.g. solenopsins in fire ants); indeed, they are barely mentioned in two reviews on ant venoms by Schmidt (1986) and recently by Touchard *et al.* (2016). However, Blum and Hermann (1978) described dolichoderine ants’ venoms as representing “one of the most variegated exocrine arsenals produced by any group of invertebrates” and attributed the success of dolichoderines in exploiting a multitude of habitats to their chemical arsenal.

Kumschick *et al.* (2015) suggested that one aspect that has not been adequately explored is whether the impacts of alien species are similar to those of species that are phylogenetically or functionally related; these authors stated that this relationship is often assumed but has rarely been tested. In the case of iridomyrmecin, one could explore whether it is present in other species and whether its presence and quantity contribute to invasive traits in those species. For example, iridomyrmecin is present in other dolichoderine ants like *Tapinoma* cf. *nigerrimum*, but it has not been detected in a closely related invasive species, *Tapinoma melanocephalum* (Appendix 1 in Section 2). Clearly these hypothesis merit further research, but even if this iridolactone is shared among members of the Dolichoderinae, it seems that this phylogenetic similarity may not be a key factor determining invasiveness in these ants.

Outlook

Altogether, my results describe various negative impacts of the Argentine ant on vertebrates. With the results of this thesis in hand, and due to the subtlety of Argentine ant venom compared to that of other invasive ants (Allen *et al.* 2004; Wetterer and Porter 2003), I can argue that, for native vertebrates in search of habitats for foraging and breeding, sites invaded by Argentine ants may be deathtraps. We should not be surprised to find negative effects of this venom on other native vertebrates such as reptiles, mammals, and birds. Since this topic is *terra incognita*, special attention should be paid to areas and seasons where vertebrates are at their most vulnerable stages (i.e. hatching, breeding periods) and are coexisting with a successful Argentine ant invasion, that is, high densities of invasive ants at their peak of activity. The latter phenomenon tends to occur in spring in temperate climates because it is related to increasing temperatures and the beginning of the reproductive cycle (Abril *et al.* 2007; Passera 1994). Furthermore, Argentine ants may be more likely to attack vertebrates in spring and autumn, when they have brood and there is an increase in demand for protein (Abril *et al.* 2007), as occurs with the red imported fire ant (Allen *et al.* 2004; Drees 1994).

The present thesis focuses on ecosystem responses to biological invasions. My results show that native vertebrates respond negatively to Argentine ant invasion, both directly and indirectly. In particular, the vertebrates affected were from the most vulnerable stages of birds and amphibians, which could result in consequences at population and community levels that scale to the ecosystem level. Finally, this thesis reveals unexpected effects of this invasive species that, although subtle, have lethal and sublethal consequences. My findings open a new door to improving our understanding of biological invasions and applying this knowledge to biological conservation.

Conclusions

1. The Argentine ant (*Linepithema humile*) disrupts the native ant community, causing indirect effects at higher trophic levels. The native amphibian community shifts to non-ant arthropod prey in invaded areas at Doñana National Park.
2. In the Doñana study area, the native amphibian species with a higher degree of ant-specialization, the natterjack toad (*Epidalea calamita*), seemed to be negatively affected by the presence of Argentine ants, as indicated by its lower abundance in invaded versus uninvaded sites and its lower feeding preference for the invasive ant.
3. The invasive Argentine ant acts as both prey and predator of newly metamorphosed amphibians of different species. The ant's venom, iridomyrmecin, is the mechanism used to subdue the prey. It is absorbed through the skin to immobilized and kill the toadlets.
4. The venom of the Argentine ant shows no signs of toxicity to natterjack toadlets when consumed orally, at least at the doses tested, which may explain why these toadlets do feed on Argentine ants. However, living in invaded areas and feeding on these ants put toadlets at risk of external envenomation and was shown to decrease survival in natterjack toadlets, and hinder growth in both natterjack and spadefoot toadlets (*Pelobates cultripes*).
5. The great tit (*Parus major*) population exhibits an apparent aversion to breeding in sites invaded by Argentine ants, and although reproductive success remained apparently unaffected, chicks reared in invaded sites suffered from poorer physical condition and altered blood parameters with respect to those reared in uninvaded sites.
6. Subtle and indirect effects of Argentine ant invasion have important implications at individual, population, community, and ecosystem levels. Native vertebrates were negatively affected at vulnerable stages but were also observed to modify their trophic ecology, spatial distribution, and habitat use in invaded areas compared to uninvaded areas. Some of the mechanisms of impact identified include prey displacement, direct predation, and sublethal effects.

Appendices

Appendix 1. Context in which iridomyrmecin appears in previous Literature. (**a**) The functions for iridomyrmecin at the first mention in the text. Some studies refer to more than one function, so proportions here are referred to the total number of functions (138). (**b**) Main goal of the article. Data come from 116 articles expanding from 1948 to 2018. (**c**) Other animal taxa having and using iridomyrmecin.

a. Function of iridomyrmecin	%
No function specified (NS)	33
Defense (DEF)	22
Insecticide (INS)	22
Antibiotic (ANT)	7
Antibacterial (AntB)	6
Alarm (AL)	4
Trail (TR)	3
Cat attracting chemical (CA)	3
Necrophoresis (NE)	1

b. Main goal of the article	%
Synthesis of iridomyrmecin (SYN)	28
Iridomyrmecin in other species (OtSp)	15
Chemical composition of exocrine secretions (ExS)	14
Chemical structure (CH)	13
Defensive compound (DEF)	9
Trail pheromone (TR)	6
Pharmacologic research (PH)	5
Insecticide (INS)	5
Antibiotic (ANT)	4
Necrophoresis (NE)	1
Alarm pheromones (AL)	1

c. Iridomyrmecin in other animal taxa		
Ant	Dolichoderinae	*Conomyrma* sp. (Cono)
		Dolichoderus scabridus (Dsca)
		Iridomyrmex nitidiceps (Inip)
		Iridomyrmex pruinosus (Ipru)
		Iridomyrmex purpureus (Ipur)
		Tapinoma erraticum (Terr)
		Tapinoma cf. *nigerrimum* (Tnig)
		Tapinoma sessile (Tsess)
		Tapinoma simrothi (Tsim)
	Myrmicinae	*Pheidole biconstricta* (Pbic)
Non-ant	Athicid beetle	*Formicomus pedestris* (Fped)
		Formicomus rubricollis (Frub)
		Microhoria terminate (Mter)
	Parasitic wasp	*Alloxysta brevis* (Abre)
		Alloxysta victrix (Avic)
		Aphidius uzbekistanicus (Auzb)
		Leptopilina heterotoma (Lhet)

Year	Reference	a	b	c
2018	Pfeiffer, L; Ruther, J; Hofferberth, J;Stökl, J. Interference of chemical defence and sexual communication can shape the evolution of chemical signals. Scientific Reports 8 (321)	DEF	OtSp	Lhet
2018	Welzel, KF; Lee, SH; Dossey, AT; Chauhan, KR; Choe, D-H. Verification of Argentine ant defensive compounds and their behavioral effects on heterospecific competitors and conspecific nestmates. Scientific Reports 8 (1477)	DEF	DEF	
2017	Bol, S; Caspers, J; Buckingham, L; Anderson-Shelton, GD; Ridgway, C; Buffington, CAT; Schulz, S; Bunnik, EM. Responsiveness of cats (Felidae) to silver vine (*Actinidia polygama*), Tatarian honeysuckle (*Lonicera tatarica*), valerian (*Valeriana officinalis*) and catnip (*Nepeta cataria*). BMC Vet Res 13:1–15	CA	OtSp	
2016	Adachi, M; Miyazawa, Y; Nishikawa, T. Improved Syntheses of (+)-Iridomyrmecin and (-)-Isoiridomyrmecin, Major Components of Matatabilactone. Nat Product Commun 11:883–886	CA	SYN	
2016	Lin, L; Cheng, XL; Li, MZ; Wang, T; Dong, MH; Wang, ZY; Liao, M. Antitumor effects of iridomyrmecin in HeLa cervical cancer cells are mediated via apoptosis induction, loss of mitochondrial membrane potential, cell cycle arrest and down-regulation of PI3K/Akt and up-regulation of lncRNA CCAT2 expression. Bangladesh J Pharmacol 11:856–862	NS	PH	
2016	Rehova, L; Dracinsky, M; Jahn, U. A general approach to iridoids by applying a new Julia olefination and a tandem anion-radical-carbocation crossover reaction. Org Biomol Chem 14:9612–9621	CA	OtSp	
2016	Scaffidi, A; Algar, D; Bohman, B; Ghisalberti, EL; Flematti, G. Identification of the Cat Attractants Isodihydronepetalactone and Isoiridomyrmecin from *Acalypha indica.* Aust J Chem 69:169–173	CA	OtSp	
2016	Stökl, J; Herzner, G. Morphology and ultrastructure of the allomone and sex-pheromone producing mandibular gland of the parasitoid wasp *Leptopilina heterotoma* (Hymenoptera: Figitidae). Arthropod Struct Dev 45:333–340	DEF	OtSp	Lhet
2015	Ebrahim, SAM; Dweck, HKM; Stokl, J; Hofferberth, JE; Trona, F; Weniger, K; Rybak, J; Seki, Y; Stensmyr, MC; Sachse, S; Hansson, BS; Knaden, M. *Drosophila* Avoids Parasitoids by Sensing Their Semiochemicals via a Dedicated Olfactory Circuit. PLoS Biol 13: e1002318	DEF	OtSp	Lhet
2015	Neff, RR. Identification and characterization of trail pheromones and queen pheromones in the Argentine ant, *Linepithema humile*. PhD thesis - University of California - Riverside, 164 pp	NS	TR	
2015	Stökl, J; Machacek, Z; Ruther, J. Behavioural flexibility of the chemical defence in the parasitoid wasp *Leptopilina heterotoma*. Sci Nat-Heidelberg 102:1–4	DEF	OtSp	Lhet
2014	Cerdá, X; van Oudenhove, L; Bernstein, C; Boulay, RR. A list and some comments about the trail pheromones of ants. Nat Product Commun 9:1115–1125	TR	TR	Tnig
2014	Chauhan, KR; Schmidt, W. Biorational synthesis of iridomyrmecin diastereomers from catnip oil. Tetrahedron Lett 55:2534–2536	DEF	SYN	
2013	Fischman, CJ; Adler, S; Hofferberth, JE. Divergent Diastereoselective Synthesis of Iridomyrmecin, Isoiridomyrmecin, Teucrimulactone, and Dolicholactone from Citronellol. J Org Chem 78:7318–7323	NS	SYN	
2013	Weiss, I; Rossler, T; Hofferberth, J; Brummer, M; Ruther, J; Stokl, J. A nonspecific defensive compound evolves into a competition avoidance cue and a female sex pheromone. . Nat Commun 4: 2767	DEF	OtSp	Lhet
2012	Choe, DH; Villafuerte, DB; Tsutsui, ND. Trail Pheromone of the Argentine Ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae). PLoS One 7: e45016	TR	TR	
2012	Hilgraf, R; Zimmermann, N; Lehmann, L; Troger, A; Francke, W. Stereoselective synthesis of trans-fused iridoid lactones and their identification in the parasitoid wasp *Alloxysta victrix*, Part II: Iridomyrmecins in the parasitoid wasp *Alloxysta victrix*. Beilstein J Org Chem 8:1256–1264	ANT	OtSp	Avic
2012	Stökl, J; Hofferberth, J; Pritschet, M; Brummer, M; Ruther, J. Stereoselective chemical defense in the *Drosophila* parasitoid *Leptopilina heterotoma* is mediated by (-)-Iridomyrmecin and (+)-Isoiridomyrmecin. J Chem Ecol 38:331–339	DEF	OtSp	Lhet
2012	Van Oudenhove, L; Boulay R; Lenoir A; Bernstein C; Cerdá X. Substrate temperature constrains recruitment and trail following behavior in ants. J Chem Ecol 38:802–809	NS	TR	Tnig

2012	Zimmermann, N; Hilgraf, R; Lehmann, L; Ibarra, D; Francke, W. Stereoselective synthesis of trans-fused iridoid lactones and their identification in the parasitoid wasp *Alloxysta victrix*, Part I: Dihydronepetalactones. Beilstein J Org Chem 8:1246–1255	NS	SYN	Avic
2011	Martinez, MJ; Weis, EM. Field observations of two species of invasive ants, *Linepithema humile* Mayr, 1868 and *Tetramorium bicarinatum* Nylander, 1846 (Hymenoptera: Formicidae), at a suburban park in Southern California. Pan-Pac Entomol 87(1):57-61	INS	DEF	
2009	Choe, DH; Millar, JG; Rust, MK. Chemical signals associated with life inhibit necrophoresis in Argentine ants. P Natl Acad Sci USA 106:8251–8255	NE	NE	
2008	Morgan, ED. Chemical sorcery for sociality: Exocrine secretions of ants (Hymenoptera: Formicidae). Myrmecol News 11:79–90	ANT	ExS	
2008	Riddick, EW; Brown, AE; Chauhan, KR. Harmonia axyridis adults avoid catnip and grapefruit-derived terpenoids in laboratory bioassays. B Insectol 61:81–90	DEF	OtSp	
2007	Lu, Y; Zhao, YP; Wang, ZC; Chen, SY; Fu, CX. Composition and antimicrobial activity of the essential oil of *Actinidia macrosperma* from China. Nat Prod Res 21:227–233	NS	OtSp	
2006	Chang, MY; Hsu, RT; Lin, CY; Chen, BF; Lin, ST; Chang, NC. Formal synthesis of (+/-)-hop ether, (+/-)-isoboonein, and (+/-)-iridomyrmecin. Heterocycles 68:271–282	AL, DEF	SYN	
2006	Schollhorn, B; Mulzer, J. Stereocontrolled formation of three contiguous stereogenic centers by free radical cyclization – Synthesis of (+)-iridomyrmecin and (-)-isoiridomyrmecin – Formal synthesis of delta-skythantine. Eur J Org Chem 2006 (4):901–908	ANT	SYN	
2006	Zhao, YP; Wang, XY; Wang, ZC; Lu, Y; Fu, CX; Chen, SY. Essential oil of *Actinidia macrosperma*, a catnip response kiwi endemic to China. J Zhejiang Univ – Sc B 7:708–712	NS	OtSp	
2005	Davidson, DW; Clark, DA; Jones, TH. Gastral exocrine products of a myrmicine ant strongly overlap pygidial gland products of Dolichoderinae. Insect Soc 52:305–308	AL, DEF	ExS	Pbic
2000	Petersen, G; Matthiesen, C; Francke, W; Wyss, U. Hyperparasitoid volatiles as possible foraging behaviour determinants in the aphid parasitoid *Aphidius uzbekistanicus* (Hymenoptera: Aphidiidae). Eur J Entomol 97:545–550	DEF	OtSp	Auzb
1999	Hodgson, DM; Gibbs, AR; Drew, MGB. Mechanism and applications of lithium amide-induced asymmetric rearrangements of 4-substituted and 4,4-disubstituted cyclopentene oxides to cyclopentenols. J Chem Soc Perk T 1 1999: 3579–3590:	NS	SYN	
1998	Billen, J; Morgan, ED. Pheromone Communication in social insects: sources and secretions. In "Pheromone Communication in Social Insects: Ants, Wasps, Bees and Termites (Vander Meer RK, Breed MD, Espelie KE, Winston ML, eds) Westview Press, Boulder, pp 3-33	AL	ExS	
1998	Horikawa, T; Norimine, Y; Tanaka, M; Sakai, K; Suemune, H. Synthesis of optically active 3ustral[3.3.0]octane skeleton using transannular reaction. Chem Pharm Bull 46:17–21	NS	SYN	
1997	Chiu, JY; Chiu, CT; Chang, NC. Total synthesis of (+/-)-patriscabrol and (+/-)-boschnialactone. J Chin Chem Soc– Taip 44:59–63	NS	SYN	
1997	Hemp, C; Dettner, K. Morphology and chemistry of mesothoracic glands in anthicid beetles (Coleoptera : Anthicidae). Entomol Gen 22:97–108	DEF	OtSp	Fges, Fped, Mter
1997	Hodgson, DM; Gibbs, AR. An enantioselective epoxide rearrangement – Claisen rearrangement approach to prostaglandins and (+)-iridomyrmecin. Synlett 1997 (6):657–658	NS	SYN	
1997	Nangia, A; Prasuna, G; Rao, PB. Synthesis of cyclopenta[c]pyran skeleton of iridoid lactones. Tetrahedron 53:14507–14545	DEF, INS, ANT	SYN	
1997	Stepanov, AV; Veselovsky, VV. Stereocontrolled synthesis of (+)- and (-)-iridomyrmecin from citronellene enantiomers. Russ Chem Bull 46:1606–1610	NS	SYN	
1996	Nangia, A; Prasuna, G. Studies on Horner-Wadsworth-Emmons reaction in base sensitive ketones: Synthesis of (-)-mitsugashiwalactone and formal synthesis of (+)-iridomyrmecin, (-)-isoiridomyrmecin and (+)-teucriumlactone. Tetrahedron 52:3435–3450	NS	SYN	
1996	Priano, M; Pavan, M. Chemical secretions of Formicidae (Hymenoptera, Formicidae). Insect Social Life 1:173–177	NS	ExS	
1995	Ohba, M; HaneishiT; Fujii, T. Syntheses of several cyclopentano-monoterpene lactones using 1,3-dioxin vinylogous ester. Chem Pharm Bull 43:26–31	NS	SYN	

1994	Lee, E; Yoon, CH. Stereoselective favorskii rearrangement of carvone chlorohydrin – expedient synthesis of (+)-dihydronepetalactone and (+)-iridomyrmecin. J Chem Soc Chem Comm 4:479–481	NS	SYN	
1994	Völkl, W; Hübner, G; Dettner, K. Interactions between *Alloxysta brevis* (Hymenoptera, Cynipoidea, Alloxystidae) and honeydew-collecting ants: How an aphid hyperparasitoid overcomes ant aggression by chemical defense. J Chem Ecol 20:2901–2915	NS	OtSp	Abre
1993	Sakail, K. Chiral rhodium complex-catalyzed asymmetric cyclization and its application to the synthesis of natural-products. J Syn Org Chem Jpn 51:733–743	NS	SYN	
1992	Agnel, G; Owczarczyk, Z; Negishi, E. Diastereoselective zirconocene-promoted bicyclization-carbonylation of allylically methyl-substituted enynes – synthesis of (+)-iridomyrmecin. Tetrahedron Lett 33:1543–1546	NS	SYN	
1992	Kigawa, M; Tanaka, M; Mitsuhashi, H; Wakamatsu, T. Synthesis of iridolactones isolated from silver vine. Heterocycles 33:117–120	NS	SYN	
1992	Yokoyama, Y; Tsuchikura, K. Doubly allylic strain – controlled diastereoselective intramolecular Michaell addition and a synthesis of (+/-)-iridomyrmecin. Tetrahedron Lett 33(20):2823–2824	NS	SYN	
1991	Simon, T; Hefetz, A. Trail-following responses of *Tapinoma simrothi* (Formicidae, Dolichoderinae) to pygidial gland extracts. Insect Soc 38:17–25	TR, AL	TR	Tsim
1989	Wang, TF; Yang, CF. Baeyer-Villiger oxidation of bicycloheptanone to cyclopentapyranone – a novel synthesis of iridomyrmecin, isoiridomyrmecin, and boschnialactone. J Chem Soc Chem Comm 24:1876–1878	DEF	SYN	
1987	Tomalski, MD; Blum, MS; Jones, TH; Fales, HM; Howard, DF; Passera, L. Chemistry and functions of exocrine secretions of the ants *Tapinoma melanocephalum* and *Tapinoma erraticum.* J Chem Ecol 13:253–263	AL, DEF	ExS	Terr
1986	Oppolzer, W;Jacobsen, EJ. Enantioselective syntheses of (+)-alpha-skytanthine, (+)-delta-skytanthine and (+)-iridomyrmecin by an intramolecular magnesium-ene reaction. Tetrahedron Lett 27:1141–1144	NS	SYN	
1984	Attygalle, AB; Morgan, ED. Chemicals from the glands of ants. Chem Soc Rev 13:245–278	INS	ExS	Inip, Ipru, Dsca
1984	Cavill, GWK; Robertson, PL; Brophy, JJ; Duke, RK; McDonald, J; Plant, WD. Chemical ecology of the meat ant, *Iridomyrmex purpureus* sens. Strict. Insect Biochem 14:505–513	NS	ExS	Ipur
1983	Hefetz, A; Lloyd, HA. Identification of new components from anal glands of *Tapinoma simrothi pheonicium.* J Chem Ecol 9:607–613	NS	ExS	Tsim
1982	Cavill, GWK; Robertson, PL; Brophy, JJ; Clark, RD; Orton, CJ; Plant, WD. Defensive and other secretions of the Australian cocktail ant, *Iridomyrmex nitidiceps.* Tetrahedron 38:1931–1938	DEF	DEF	Inip
1981	Blum, MS. Chemical defenses of arthropods. 9. Lactones, pp. 239-246. Academic Press, Inc (London). ISBN 0-12-108380-2	INS	DEF	
1981	Grieco, PA; Srinivasan, CV. Stereochemical consequence of the coupling of lithium dimethylcuprate with a cyclopentenyl allylic lactone - total synthesis of dl-iridomyrmecin. J Org Chem 46:2591-2593	NS	SYN	
1981	Van Vorhis Key,SE; Gaston, LK; Baker, TC. Effects of gaster extract trail concentration on the trail following behaviour of the Argentine ant, *Iridomyrmex humilis (Mayr). J Insect Physiol 27:363–370*	INS	TR	
1980	Cavill, GWK; Davies, NW; McDonald, FJ. Characterization of aggregation factors and associated compounds from the Argentine ant, *Iridomyrmex humilis.* J Chem Ecol 6:371–384	DEF	ExS	
1980	El-Naggar, LJ; Beal JL. Iridoids. A review. Journal of Natural Products 43(6):649-707	DEF	ExS	
1979	Smith, RM; Brophy, JJ; Cavill, GWK; Davies, NW. Iridials and nepetalactone in the defensive secretion of the coconut stick insects, *Graeffea crouani.* J Chem Ecol 5:727–735	NS	OtSp	
1978	Blum, MS; Hermann, HR. Venoms and venom apparatuses of the Formicidae: Dolichoderinae and Aneuretinae. In Arthropod venoms, pp 871-894. Ed Bettini Handbook of Experimental Pharmacology	INS	DEF	
1978	Yamada, Y; Sanjoh, H; Iguchi, K. Synthesis of (+/-)-iridomyrmecin from the biogenetic precursor. Chem Lett 7:1405–1406	NS	SYN	
1977	Wheeler JW, Olagbemiro T, Nash A, Blum MS. Actinidine from the defensive secretions of dolichoderine ants. J Chem Ecol 3:241–244	DEF	DEF	Cono
1976	Cavill, GWK, Houghton, E, McDonald, FJ, Williams, PJ. Isolation and characterisation of dolichodial and related compounds from the Argentine ant, *Iridomyrmex humilis.* Insect Biochem 6:483–490	DEF	ExS	

1975	Lieberburg, I; Kranz, PM; Seip, A. Bermudian ants revisited: the status and interaction of *Pheidole megacephala* and *Iridomyrmex humilis*. Ecology 56(2):473-478	INS	DEF	
1975	Matthews, RS; Whitesell, JK. Transannular cyclizations. Steroselective synthesis of cyclopentanoid monoterpenes. J Org Chem 40(22):3312-3313	INS	SYN	
1974	Cavill GWK, Houghton E. Volatile constituents of the Argentine ant, *Iridomyrmex humilis*. J Insect Physiol 20:2049–2059	DEF	ExS	
1971	Cavill, GWK; Clark, DV. Ant secretions and cantharidin. In Naturally occurring insecticides. pp. 271-305. Ed Marcel Dekker	INS	INS	
1970	Cavill, GWK. Chemistry of some insect secretions. Liversidge Research Lecturer No. 18 – School of Chemistry, The University of New South Wales, Kensington, N.S.W. 2033. Pp 5-18	INS, DEF	ExS	
1969	Blum, MS. Alarm pheromones. Annu Rev Entomol 14:57–80	AL	AL	
1968	McGurk, DJ; Frost, J; Waller, GR; Eisenbraun, EJ; Vick, K; Drew, WA; Young, J. Iridodial isomer variation in Dolichoderine ants. J Insect Physiol 14:841–845	DEF	CH	Ipru
1967	Cavill, GWK, Clark, DV. Insect venoms, attractants, and repellents – VIII. Isidihydronepetalactone. J Insect Physiol 13:131–135	DEF	ExS	
1967	Overberber, GC; Kaye, H. Optical rotatory dispersion and conformtion of some optically active epsilon-caprolactones. J Am Chem Soc 89(22):5646-5649	NS	CH	
1966	Eisner, T; Meinwald, J. Defensive secretions of Arthropods. Science 153:1341–1350	DEF	DEF	
1965	Sakan, T; Isoe, S; Hyeon, SB, Katsumura, R; Maeda, T; Wolinsky, J; Dickerson, D; Slabaugh, M; Nelson, D. The exact nature of matatabilactone and the terpenes of *Nepeta cataria*. Terahedrom Lett 6(46):4097-4102	NS	CH	
1965	Wolinsky, J; Gibson, T; Chan, D; Wolf, H. Stereospecific syntheses of iridomyrmecin and related iridolactones. Tetrahedron 21:1247–1261	DEF, INS, ANT	SYN	
1964	McConnell, JF; Mathieson, AM; Schoenborn, BP. The crystal structure of monoterpene iridomyrmecin at -150ºC. Acta Cryst 17:472–477	NS	CH	
1964	Sisido, K; Isida, T; Utimoto, K. A synthesis of iridomyrmecin. J Org Chem 29:3361–3365	NS	SYN	
1963	Falbe, J; Weitkamp, H; Korte, F. Zur synthese bicyclischer lactone vom typ des D,L-iridomyrmecins. Tetrahedron 19:1479–1482	NS	SYN	
1962	Roth, LM, Eisner, T. Chemical defences of arthropods. Annu Rev Entomol 7:107–136	DEF, INS	DEF	
1962	Korte, F; Kochen, W; Ludwig, G; Rechmeier G; Schreiber HJ; Stiasni M; Vogel J . 14C-markierte Insektizide aus der Reihe der halogenierten Kohlenwasserstoffe und des Iridomyrmecins. Angew Chem – Ger Edit 74:503	INS	INS	
1962	Korte, F; Schreiber HJ. Insektizide im Stoffwechsel, IV. Iridomyrmecin-[3-14C]. Liebigs Ann Chem 656:145–148	INS	SYN	
1962	McConnell, JF; Mathieson, AM; Schoenborn, BP. Conformation of iridomyrmecin and isoiridomyrmecin. Tetrahedron Lett 10:445–448	NS	CH	
1961	Cavill, GWK; Hinterberger, H. The Chemistry of Ants. V. Structure and Reactions of Dolichodial. Aust J Chem 14:143–149	NS	CH	
1961	Dolejs, L; Mironov, A; Sorm, F. On terpenes .121. Structure of bulnesol and stereochemistry of guaiol, nepetalinic acids and iridomyrmecins. Collect Czech Chem Comm 26:1015–1020	NS	CH	
1960	Cavill, GWK. The cyclopentanoid monoterpenes. Reviews of Pure and Applied Chemisty. 10(3):169-183	INS	ExS	
1960	Cavill, GWK; Hinterberger, H. The chemistry of ants. IV. Terpenoid constituents of some *Dolichoderus* and *Iridomyrmex* species. Aust J Chem 13:514–519	NS	CH	Dsca
1960	Dolejs, L; Mironov, A; Sorm, F. Structure of bulnesol stereochemistry of guaiol, nepetalinic acids and iridomyrmecins. Tetrahedron Lett 11:18–21	NS	CH	
1959	Clark, KJ; Fray, GI; Jaeger, RH; Robinson, R. Synthesis of D- and L- isoiridomyrmecin and related compounds. Tetrahedron 6:217–224	NS	SYN	
1959	Jaeger, RH; Robinson, R. The conversion of Deuterium-isoiridomyrmecin into Deuterium-iridomyrmecin Tetrahedron Lett 1(15):14-18	NS	CH	
1959	Korte, F; Falbe, J; Zschocke, A. Alpha-hydroxyalkyliden-lacton-umlagerung .9. Synthese des D,L-Iridomyrmecins und verwandter bicyclischer Lactone. Tetrahedron 6:201–216	INS, ANT	SYN	

1959	Sakan, T; Fujino, A; Murai, F; Busuga,n Y; Suzuui, A. The structure of Matatabilactone. Bull Chem Soc 32(10):1154-1155	NS	CH
1959	Wilson, EO; Pavan, M. Glandular sources and specificity of some chemical releasers of social behavior in dolichoderine ants. Psyche 66:70–76	TR	TR
1958	Clark, KJ; Fray, GI; Jaeger, RH; Robinson, R. Eine synthese des D-und L-Iso-Iridomyrmecins. Angew Chem – Ger Edit 70:704	NS	SYN
1958	Clark, KJ; Fray, GI; Jaeger, RH; Robinson, R. Configuration of iridodial, isoiridomyrmecin and iridomyrmecin. Chem & Industry 45:1473	NS	CH
1958	Korte, F; Falbe, J; Zschocke, A. Synthese des D,L-Iridomyrmecins und verwandter Lactone. Angew Chem – Ger Edit 70:704	NS	SYN
1958	Pavan, M; Trave, R. Études sur les Formicidae. IV Sur le venin du Dolichodéride *T. nigerrimum.* Insect Soc 5:299–308	INS, AntB	DEF
1958	Wendler, NL; Slates, HL. Studies in the Iridomyrmecin series - abnormal ring closure of a 1,6-keto aldehyde. J Am Chem Soc 80:3937–3939	NS	CH
1957	Cavill, GWK; Locksley, HD. The chemistry of ants. II. Structure and configuration of iridolactone (isoiridomyrmecin). Aust J Chem 10:352–358	NS	CH
1956	Cavill, GWK; Ford, DL; Locksley, HD. The chemistry of ants. I. Terpenoid constituents of some Australian *Iridomyrmex* species. Aust J Chem 9: 288–293	ANT, INS	ExS
1955	Fusco, R.; Trave, R.; Vercellone, I. Ricerche sull'iridomirmecina, l'insetticida naturale secreto dalla *Iridomyrmex humilis* Mayr. La Chimica e l'Industria 37(4):251–259	INS	INS
1955	Fusco R, Trave R, Vercellone A. La struttura dell'iridomirmecina. La Chimica e l'Industria 37:958-959	INS	CH
1955	Pavan, M. Sull’attività fitoinibente della iridomirmecina su *Lupinus albus*. Boll Soc Ital Biol Sper 31:967–969	ANT, AntB	PH
1955	Pavan, M; Baggini, A. Ricerche sull’attività fitoinibitrice dell’iridomirmecina su *Lupinus albus*. Boll Zool 22:393–404	INS	PH
1955	Pavan, M; Ronchetti, G. Studi sulla morfologia esterna e anatomia interna dell' operaia di *Iridomyrmex humilis* Mayr e ricerche chimiche e biologiche sulla iridomirmecina. Atti Soc It Sc Nat 94:379–477	INS, AntB	PH
1955	Pavan, M; Valcurone, ML. Antagonismo della iridomirmecina verso l’effetto oncogeno della colchicina e del gammaesano su *Lupinus albus*. Boll Soc It Biol Sper 31:969-971	DEF	PH
1952	Pavan, M. Iridomyrmecin as insecticide. IX Int Congr Entomol Amsterdam 17-24 August 1951, 1: 321–327	INS, AntB	INS
1952	Pavan, M. Primo contributo sperimentale allo studio farmacologico della iridomirmecina. Arch Int Pharm Thér 89: 223–228.	AntB, INS	PH
1952	Pavan, M. Sugli antibiotici di origine animale. II. Ricerche personali. Boll Ist Mil 31(5-6):232-245	AntB, INS	ANT
1951	Pavan, M. Sull’attivita insetticida della iridomirmecina. Mem Soc Ent It 30:107–132	INS	INS
1950	Pavan, M. Potere insetticida della iridomirmecina e significato della sostanza nella biologia di *Iridomyrmex humilis* Mayr (*Formica argentina*). La Ricerca Scientifica 20:1835–1855	INS	INS
1950	Zyka, J. New antibiotics, chloromycetin, aureomycin, and iridomyrmecin. Cas Cesk Lek 63(15):172-173	INS, AntB	ANT
1949	Pavan, M. Ricerche sugli antibiotici di origine animale. Nota riassuntiva	AntB	ANT
1948	Pavan, M; Nascimbene, A. Studi sugli antibiotici di origine animale. I. Su un principio antibiotico di *Iridomyrmex pruinosus humilis* Mayr. (nota prev.). Boll Soc Med Chir Pavia 62:193–197	ANT	ANT
1948	Pavan, M; Nascimbene, A. Studi sugli antibiotici di origine animale. X. Nuovi risultati sulla iridomirmecina . Boll Soc Med Chir Pavia 62:295–298	ANT	ANT

Oecologia (2017) 185:95–106
DOI 10.1007/s00442-017-3929-x

BEHAVIORAL ECOLOGY – ORIGINAL RESEARCH

Native predators living in invaded areas: responses of terrestrial amphibian species to an Argentine ant invasion

Paloma Alvarez-Blanco[1] · **Stephane Caut**[1] · **Xim Cerdá**[1] · **Elena Angulo**[1]

Received: 29 April 2016 / Accepted: 8 August 2017 / Published online: 22 August 2017

Abstract Predator–prey interactions play a key role in the success and impacts of invasive species. However, the effects of invasive preys on native predators have been poorly studied. Here, we first reviewed hypotheses describing potential relationships between native predators and invasive preys. Second, we examined how an invasive prey, the Argentine ant (*Linepithema humile*), affected a native terrestrial amphibian community. In the field, we looked at the structure of the amphibian community in invaded versus uninvaded areas and characterized amphibian trophic ecology. The amphibian community sampled seemed to show a species-dependent response in abundance to invasion: adults of the natterjack toad (*Bufo calamita*), the species demonstrating the highest degree of ant specialization, were less abundant in invaded areas. Although available ant biomass was significantly greater in invaded than in uninvaded areas (only Argentine ants occurred in the former), amphibians consumed relatively fewer ants in invaded areas. In the lab, we quantified amphibian consumption of Argentine ants versus native ants and assessed whether consumption patterns could have been influenced by prior exposure to the invader. The lab experiments corroborated the field results: amphibians preferred native ants over Argentine ants, and prior exposure did not influence consumption. Differences in preference explained why amphibians consumed fewer Argentine ants in spite of their greater relative availability; they might also explain why the most ant-specialized amphibians seemed to avoid invaded areas. Our results suggest the importance to account for predator feeding capacities and dietary ranges to understand the effects of invasive species at higher trophic levels.

Keywords Biotic resistance · Enemy release · Exotic prey naïveté · Invasive prey · *Linepithema humile*

Introduction

The vulnerability of native communities to invasions of non-native species depends on their ecological resistance, which is mainly defined by the presence of native competitors and predators (Ricciardi et al. 2013). Predators can promote resistance through a variety of mechanisms, including their abundance, their recognition and consumption of invasive prey, their functional response to invasive prey, and their ability to respond over time (Catford et al. 2009; Carlsson et al. 2009; Twardochleb et al. 2012; Carthey and Banks 2014).

For example, the well-known enemy release hypothesis (as well as its variants, such as the enemy reduction hypothesis and the enemy inversion hypothesis; Catford et al. 2009) states that exotic species can become invasive because they lack coevolved enemies in their introduced ranges (Keane and Crawley 2002; Colautti et al. 2004; Sih et al. 2010). In such situations, native predators do not limit the invasion, because predation does not occur or only occurs at low levels. In contrast, the more recently published exotic prey

---

Communicated by Peter Banks.

Paloma Alvarez-Blanco and Stephane Caut contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s00442-017-3929-x) contains supplementary material, which is available to authorized users.

✉ Stephane Caut
stephanecaut@gmail.com

[1] Estación Biológica de Doñana, CSIC, Avda. Americo Vespucio 26, 41092 Seville, Spain

naïveté hypothesis and the increased susceptibility hypothesis posit that the naïveté of introduced prey species means they experience higher predation pressures than do native prey species (Colautti et al. 2004; Catford et al. 2009; Li et al. 2011; Wanger et al. 2011). In this case, native predators should be able to control the invasion by predating upon the introduced prey species, acting as a form of biotic resistance. The specialist–generalist hypothesis states that invasion success should be minimized when predators are generalists because they would, thus, be able to consume introduced prey (Sax et al. 2007; Catford et al. 2009). Although dietary specialization actually exists along a gradient, Catford et al. (2009) distinguish two extremes: the absolute generalist, which interacts with any and all species, and the absolute specialist, which preys upon a single species. However, if the invasive prey species can defend itself chemically (e.g., with toxins), then it could escape even predation by generalists. Such a situation is described by the novel weapons hypothesis—the competitive ability of invasive prey would, therefore, be enhanced (Callaway and Ridenour 2004; Carlsson et al. 2009; Ricciardi et al. 2013).

These hypotheses are rather one-sided in that they largely focus on the success of the introduced prey species without addressing effects on native predators (but see Pintor and Byers 2015). By considering the effects on predators in each of these scenarios, we can establish a theoretical framework for understanding how invasive prey affects native predators. Here, we have expanded the hypotheses described above to address the effects of introduced prey on predators with different degrees of dietary specialization. According to the exotic prey naïveté and the increased susceptibility hypotheses, predators may benefit from the large availability of naïve prey because they can consume them (Glenn and Holway 2008; Wanger et al. 2011; Cabrera-Guzmán et al. 2012; Monzo et al. 2013). Based on the specialist–generalist hypothesis, generalist may benefit more than specialist predators (Maerz et al. 2005; Sax et al. 2007; Catford et al. 2009). And based on the enemy release hypothesis native predators would benefit more if the introduced prey species were relatively similar to native prey species (Carlsson et al. 2009; Robbins et al. 2013). Finally, according to the novel weapons hypothesis, predators will not benefit if the invasive prey releases toxins. Although native predators could develop ways for dealing with toxins, this process is expected to occur over the long term (Carlsson et al. 2009). Different degrees of naïveté have been observed in prey (Banks and Dickman 2007) and may also exist in native predators. In this sense, Bytheway et al. (2016) have shown how behavioral flexibility on the part of invasive predators can enable invaders to respond rapidly to novel situations. However, such behavioral flexibility should be less common in native predators (Carlsson et al. 2009): the predator may not recognize a new prey species; it may recognize an invasive species as prey but fail to capture it; or it may capture it without consuming it. Taken together, these hypotheses suggest that native predators are more likely to be negatively impacted under the following conditions: they have a more specialized diet; the invasive prey species differs from the native prey species included in their diets; or the invasive prey species releases toxins.

Ants play crucial ecological roles within ecosystems (Lach et al. 2010) and, consequently, the negative effects of invasive ants can scale up to higher trophic levels (Holway et al. 2002). The Argentine ant (*Linepithema humile*) is one of the five ant species included on the list of 100 of the world's worst invaders (Lowe et al. 2000; Luque et al. 2013). It has a remarkable ability to establish itself in natural ecosystems outside of its native range; it has had striking success in Mediterranean ecosystems all over the world (Suarez et al. 2001; Wetterer et al. 2009; Vogel et al. 2010). It has been used to examine a variety of ecological issues across different continents (Pysek et al. 2008). For example, its negative effects on ant communities have been studied in the greatest detail in California and Europe (e.g., Carpintero et al. 2005; Gordon and Heller 2014). Once the Argentine ant has established itself, it displaces almost all native ant species (Suarez et al. 1998; Carpintero et al. 2005; Holway and Suarez 2006; Angulo et al. 2011). Its effects on non-ant species, including ant specialist predators, have been studied in California and Japan (e.g., Suarez and Case 2002; Touyama et al. 2008). Unlike other invasive ants (e.g., *Solenopsis invicta*, *Wasmania auropunctata*), which have a venomous sting, the Argentine ant does not possess a functional stinger that it could use to defend itself from predators or to subdue vertebrate prey (Holway et al. 2002). Although the Argentine ant can prey on nestlings of some bird species, it has not been considered to be a serious threat (Sockman 1997; Hooper-Bui et al. 2004; Suarez et al. 2005; Estany-Tigerström et al. 2010, 2013). When it comes to native predators in general, negative effects related to prey displacement have been observed for the ant-eating specialist *Phrynosoma coronatum*, the coastal horned lizard (Suarez et al. 2000; Suarez and Case 2002); conversely, some ant-eating invertebrates appear to benefit from the presence of this invasive ant (Touyama et al. 2008; Glenn and Holway 2008). These contrasting results suggest that dietary specialization is not the only factor driving the impacts of the Argentine ant on predators.

In this study, we examined the threat posed by the Argentine ant to a native amphibian community. Amphibians are the world's most vulnerable group of vertebrates (accounting for ~41% of endangered species; Hoffmann et al. 2010). Terrestrial amphibians are known to consume large quantities of ants (see Online Resource 1) and are, thus, potentially vulnerable to Argentine ant invasions. We aimed to answer three key questions. First, is amphibian abundance different in invaded areas because it is dependent on dietary specialization? We predicted that the greater a species' dietary specialization on ants, the more its abundance would decrease in invaded areas. Second, is ant availability similar in invaded and uninvaded areas, and do predators with different dietary specializations track ant availability differently? We predicted that ant consumption would track ant availability for generalist predators but would decrease for the most specialized predators. Because no other ant species are available in invaded areas, predators could compensate by consuming prey of other taxa (i.e., by excluding ants from their diets). Then, if a dietary shift was to occur, the predators' nitrogen isotopic values would be expected to differ because the nitrogen isotopic value of a predator reflects that of its prey (Post 2002). Third, do amphibian ant specialists prefer native ants to Argentine ants? Is this preference affected by ant morphology or by prior exposure to the Argentine ant? We predicted that the consumption of Argentine ants by amphibian ant specialists would depend on the degree of similarity between the Argentine ant and the native ant species consumed by the specialist. We also predicted that prior exposure to the invader could alter consumption patterns in one of two ways: (a) consumption could increase relative to a naïve individual if the encounter resulted in the amphibian learning to recognize the Argentine ant as prey or (b) consumption could decrease relative to a naïve individual if the prior exposure resulted in a negative experience and the amphibian learned to avoid eating Argentine ants.

Methods

Field study

Study area

The field study was conducted in the Doñana Biological Reserve (37°1′N, 6°33′W; Doñana National Park, Spain) in an open Mediterranean scrubland containing scattered pine (*Pinus pinea*) forests and isolated cork oak trees (*Quercus suber*). One week of sampling was conducted during the summer and fall of 2009 and the winter and spring of 2010.

The reserve is home to more than 30 native ant species. The Argentine ant arrived at Doñana in the 1970s at the reserve's field station (Angulo et al. 2011). Given that queens are wingless and workers travel only short distances (Heller et al. 2008), the invasion of natural areas relies on inadvertent and sporadic transport by humans, predators, or scavengers (Carpintero et al. 2005). Because the species avoids the scrubland (due to its low tolerance of high temperatures and dry habitats), it is now found in individual cork oaks and pine forests (Angulo et al. 2011). The close association between the ants and the cork oaks is fostered by food availability. Under the cork oak canopies, a dense network of interconnected nests can be found, and ants also forage in the tree trunk and branches (Carpintero et al. 2005). The cork oak is a keystone species because it shelters many species against the region's hot, dry summers—the tree's canopy provides shade and the root system keeps shallower soil levels humid (Kurz-Benson et al. 2006)—and its location near temporary ponds results in a clear environmental gradient under the tree canopy (wetter conditions closer to the pond side and drier conditions on the opposite side of the tree).

As amphibians live around ponds, they are likely to interact with Argentine ants from invaded cork oaks. The most abundant terrestrial species are the natterjack toad (*Bufo calamita*), the western spadefoot toad (*Pelobates cultripes*), the Mediterranean treefrog (*Hyla meridionalis*), and the Iberian painted frog (*Discoglossus galganoi*) (Díaz-Paniagua et al. 2010). Although none of them are absolute ant specialists (Online Resource 1), we can order them according to the percentage of their diet that is represented by ants: *B. calamita* (up to 72%) > *H. meridionalis* (up to 58%) > *D. galganoi* (up to 17%) > *P. cultripes* (up to 4%).

Sampling took place in and under ten centenarian cork oaks (hereafter, tree areas), five of which had been invaded and five of which remained uninvaded by the Argentine ant. Each tree area was treated as an independent replicate. To be more certain that the amphibians studied were not experiencing both invaded and uninvaded areas, no invaded tree area was closer than 250 m to any uninvaded tree area (and vice versa). Within groups (invaded or uninvaded), tree areas were separated by at least 40 m. This distance guaranteed independence in ground and tree arthropod sampling (Angulo et al. 2007; Gove et al. 2009). We were only able to sample a limited number of trees because the National Park restricted amphibian trapping and the access to some invaded trees (because of waterbird conservation concerns) and because the Argentine ant invasion pattern is patchy.

Sampling the abundance of amphibian predators

Amphibians were captured using three pitfall trap lines composed of three bucket traps each (30 × 40 cm) and a barrier of 3 m × 50 cm to guide individuals into the buckets (Fig. 1). Traps were deployed for 7 days during each season and checked every 3 h. Most were euthanized to examine their gut contents and to collect tissue samples for the stable isotope analyses. Samples were kept in 70% alcohol until further analyses could take place in the laboratory.

Sampling the availability of invertebrate prey

Invertebrates were sampled using seven pitfall traps (200-ml PVC cups 2/3 full of soapy water) and two white traps (for

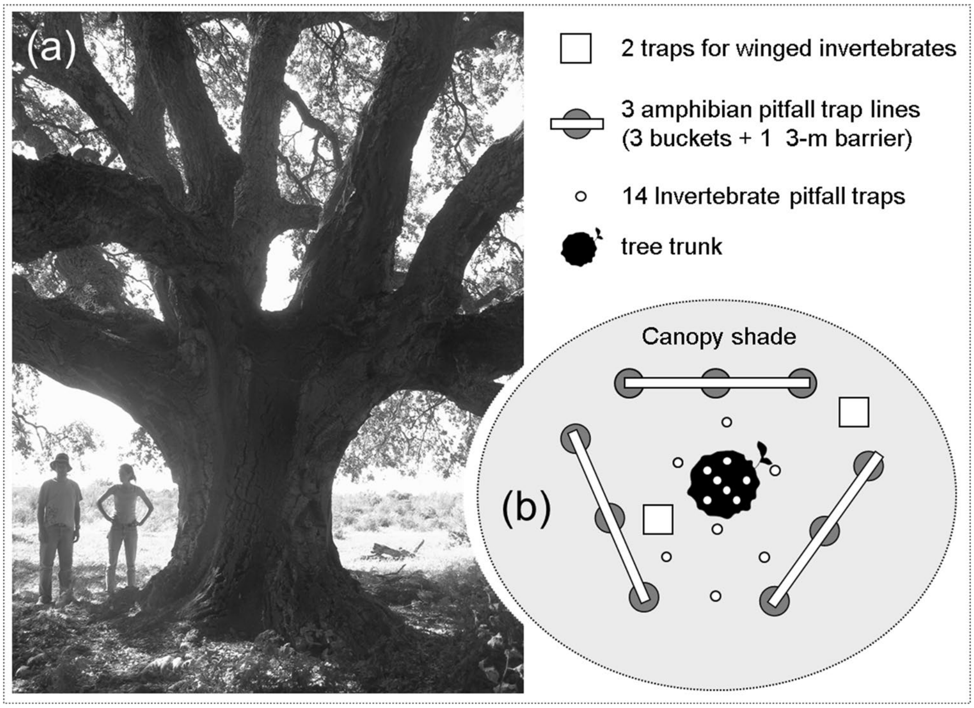


Fig. 1 **a** A picture of a cork oak (*Quercus suber*) and **b** schematic of the trapping protocol for a given tree. The pitfall traps shown inside the tree trunk were attached to the trunk and branches

flying arthropods; 25 × 50 × 10 cm trays partially filled with soapy water) placed on the ground under the tree canopy (Fig. 1). Another seven pitfall traps were attached to the tree's branches and trunk. Traps were deployed for 3 days per sampling period and the invertebrates, collected every day, were kept in 70% alcohol.

All invertebrates were identified to the lowest taxonomic level possible. The total number of individuals was then calculated for each taxonomic group caught for each sampling day, tree area, and sampling season. This number was used to estimate biomass: the number of individuals was multiplied by the mean mass for each taxonomic group, which was obtained by measuring the dry mass of 10–30 individuals.

Stomach content analyses

Predator stomach fullness (i.e., whether the stomach contained food or was empty) was determined, and the stomach contents were removed and preserved in alcohol (70%) until the prey species could be identified to the lowest taxonomic level possible using the invertebrates obtained in the pitfall traps as references. The relative importance of each prey item in a predator's diet was assessed in two ways: (1) using prey biomass: the percentage of total biomass attributable to each prey item (100 × [biomass of a specific prey item/total biomass of all prey items]) and (2) prey frequency: the percentage of each prey item across all non-empty stomachs (100 × [number of stomachs containing a specific prey item/total number of stomachs containing prey]). To limit bias due to digestion in the biomass calculations, the mass of the whole body of one individual was used, which was determined using the individuals obtained via pitfall trapping.

A cumulative prey curve was constructed to assess whether an adequate number of stomachs had been sampled. The order of the stomachs was randomized ten times, and the mean (±SE) of singleton prey items was plotted to minimize the possible bias resulting from sampling order. The point at which the prey curve approached an asymptote revealed the number of stomachs needed to accurately characterize the diet (Online Resource 2).

Isotopic analyses

Stable isotope methods are currently among the most powerful tools used in the study of trophic relationships and animal diets. However, it is difficult to obtain exact estimates of isotopic values, as they can be affected by a number of factors (Post 2002; Vanderklift and Ponsard 2003; Caut et al. 2009). To reduce variability when comparing the isotopic values of amphibians captured in different areas and on different dates, it is necessary to obtain an isotopic baseline (Lorrain et al. 2014). Isotopic baselines are known to vary across time and space and can influence the range of nitrogen isotopic values within a given food chain at a given time. To estimate the nitrogen isotopic baseline, samples of the most abundant plant species found in the shade cast by the canopy of each tree were collected during each season and identified. The mean of their isotopic values was used as the baseline. To estimate the nitrogen isotopic values for the amphibians, liver samples were collected. Both sample types (plants and amphibian livers) were dried at 60 °C for 48 h, ground to a fine powder, weighed in tin capsules, and stored in a desiccator until isotopic analyses took place. The analyses were performed using a continuous flow isotope ratio mass spectrometry system that consisted of a Flash HT Plus elemental analyser coupled to a Delta-V Advantage isotope ratio mass spectrometer via a CONFLO IV interface (Thermo Fisher Scientific, Bremen, Germany). The system was located in the Stable Isotope Laboratory at the Doñana Biological Station (LIE-EBD; http://www.ebd.csic.es/lie/Home.html). Isotopic ratios are presented as δ values (‰); they are relative to atmospheric nitrogen and expressed as $\delta^{15}N = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, where R is $^{15}N/^{14}N$. The reference material was IAEA-N1 (+0.4‰). Replicates of internal laboratory standards (which had been previously calibrated with international standards such as IAEA-N1) were regularly included in the sampling sequence and indicated that analytical measurement errors never exceeded ±0.2‰.

Laboratory preference experiment

Fourteen adult natterjack toads (*B. calamita)* were captured in the field in spring 2013. Eight came from uninvaded areas, and six came from invaded areas. All the adults collected in uninvaded areas were captured 1.5 km away from invaded areas. It is highly unlikely that they had previous contact with the Argentine ant. In the laboratory, they were individually housed and fed mealworms, pillbugs, and small crickets ad libitum. The day before each preference trial, the toads were not fed.

In addition, several hundred workers were collected from colonies of the Argentine ant and from colonies of three native ant species: *Tapinoma nigerrimum*, which is similar in size and taxonomically close to the Argentine ant; *Aphaenogaster senilis*, 3–10 times larger than the Argentine ant and one of the most abundant scrubland species in our study area; and *Crematogaster scutellaris*, the most abundant species in Doñana's cork oaks (Carpintero et al. 2005).

Two kinds of preference tests were performed. In the first test, five workers of each ant species (for a total of 20 ants) were simultaneously added to a terrarium. A toad was then placed in the center of the terrarium, and the time at which it ate each of the ants over a 30-min period was recorded. Each toad was tested five times ($N = 70$ trials; 14 individuals; 1400 ants tested); trials took place at least 3 h apart. In the second test, which also lasted 30 min, 20 ants of the same species were placed in a terrarium, and the time at which the toad ate the ants was recorded. Each toad was tested four times, with each of the four ant species ($N = 56$ trials; 14 individuals; 1200 ants tested). The order in which they experienced the species was random, and trials were separated by at least 18 h. Both types of tests were performed because, in the field, *L. humile* rarely co-occurs with native ants. As a consequence, amphibians will rarely have to choose between native and invasive species. However, it is nonetheless informative to quantify preferences and consumption rates under both sets of conditions.

Statistical analyses

General linear models were used to compare

(a) The number of individuals (dependent variable) of different amphibian species found in invaded versus uninvaded areas across different seasons (independent variables). When juveniles were also found, adults and juveniles were placed in two distinct categories in the "species" variable. This differentiation between adults and juveniles reflects an important spatial constraint related to amphibian biology. Juveniles are unable to choose the ponds from which they emerge, while adults can choose where they forage and breed. The model examining overall amphibian abundance included tree area (five levels), invasion status (invaded vs. uninvaded), season (four levels), and amphibian species [six levels: *B. calamita* (adults and juveniles), *P. cultripes*, *H. meridionalis*, and *D. galganoi* (adults and juveniles)]. We carried out separate analyses for each amphibian species (by specifying the "by" option in Proc Genmod, SAS software v. 9.2, SAS Institute 2008).

(b) Total available biomass and the percentage of available ant biomass (dependent variables) in invaded versus uninvaded areas across seasons (independent variables); the invasion-by-season interaction was also included. The models included tree area (five levels), invasion status (invaded vs. uninvaded), season (four levels), and sampling day (three levels). Thus, the totals used were for each day of each season and for each of the invaded and uninvaded tree areas.

(c) Total biomass in stomach contents and the percentage of ant biomass in stomach contents (dependent variables) for different amphibian species in invaded versus uninvaded areas across seasons (independent variables) (note: *D. galganoi* was excluded from these analyses because of its small sample size). In this case, the sample size was the number of individual amphibians for which stomach contents could be analyzed and were not empty ($N = 95$, see Online Resource 3a). The model included amphibian species (four levels), tree area (five levels), invasion status (invaded vs. uninvaded), and season (four levels). Thus, the totals used were for each amphibian species, for each season, and for each of the invaded and uninvaded tree areas.

(d) The nitrogen isotopic baseline ($\delta^{15}N_{TREE}$ = mean $\delta^{15}N$ of plants; dependent variable) for the different tree areas across seasons (independent variables). The model examining the baseline isotopic values included tree area (five levels) and season (four levels).

(e) The nitrogen isotopic values of amphibians ($\delta^{15}N$ of liver tissue; dependent variable) of different species in invaded versus uninvaded areas across seasons (independent variables). To standardize the comparisons, amphibian tissue $\delta^{15}N$ values were corrected using the isotopic baseline of each tree during each sampling period ($\delta^{15}N_{COR} = \delta^{15}N_{LIVER} - \delta^{15}N_{TREE}$). In this case, the sample size was the number of individual amphibians for which we obtained isotopic values ($N = 106$, Online Resource 3a). The model included amphibian species (six levels), invasion status (invaded vs. uninvaded), the invasion-status-by-season interaction, and season (four levels).

As necessary, tree area identity was included as a repeated measures factor ("repeated subject" command in Proc Genmod, SAS software v. 9.2). Models of the total stomach content biomass also included the sex of the animal as a fixed effect. The normality of all the dependent variables was tested before models were fitted. A Poisson distribution and a log-link function were used for the models of available biomass and stomach content biomass (except in the case of the total biomass of stomach contents, for which a gamma distribution with a log-link function was used); model deviance was, thus, minimized. Because one of the invaded trees was flooded during the winter and the spring, the number of invaded trees was reduced to four.

The data from the preference experiments were analyzed using survival analyses, which estimated the probability of ants being eaten as a function of time. Mixed effects Cox models were used so that random factors could be included; we employed the coxme package (Therneau 2015) in the R software (R Core Team 2016). For the first test, the model included (a) ant species, to evaluate toad preference for different prey species; (b) the invasion status of the areas where the amphibians were captured to test for differences in amphibian naïveté to the Argentine ant; (c) the trial order for a given individual, to test whether learning occurred following exposure to the ants; and (d) the ant-species-by-trial-order interaction to test for differences in amphibian learning among ant species. Because learning was ant-species dependent, trial order was also tested for each ant species separately. Individual amphibian identity and trial number were included as random factors. For the second test, the model included only ant species and individual amphibian identity (as a random factor). The significance of each variable was tested using a Chi-squared test that compared the likelihood of the full model with that of the full model minus the variable of interest. In the latter model, the interaction between two variables was also removed when the significance of only one of the two variables was being tested. When significant, the model with the highest likelihood value (or the simplest model in case this value was equal) was considered the best.

Results

Amphibian abundance in invaded and uninvaded areas

Over a total of 342 trap nights, 174 amphibians of 4 different species were caught: 124 natterjack toads (*B. calamita*), 27 western spadefoot toads (*P. cultripes*), 15 stripeless tree frogs (*H. meridionalis*), and 8 Iberian painted frogs (*D. galganoi*) (Fig. 2a). All were adults, except for most of the *B. calamita* captured in the spring (90 juveniles and 5 adults) and most of the *D. galganoi* (6 juveniles) (Online Resource 3a, Fig. 2a).

In the case of *D. galganoi* and *H. meridionalis*, the numbers of adults captured did not differ based on invasion status or season, nor was the interaction between variables significant (*D. galganoi* $\chi^2_1 = 0.03$, $p = 0.860$;

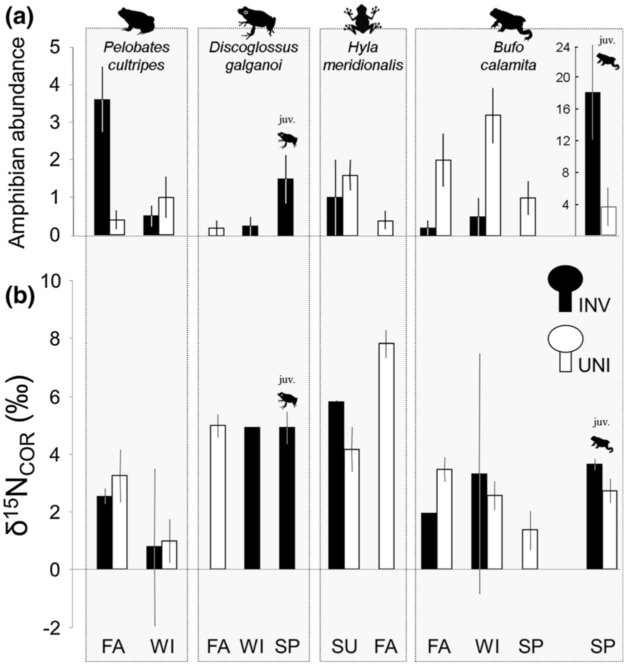


Fig. 2 **a** Number of amphibians captured (mean ± SE) and **b** the $\delta^{15}N_{COR}$ values of amphibian livers (mean ± SE) for invaded and uninvaded areas (INV in *black* and UNI in *white*, respectively) across different seasons (*SU* summer, *FA* fall, *WI* winter, *SP* spring). Data for adults and juveniles are separated (juveniles are specified with "juv."). Only seasons for which abundance was greater than zero in at least one tree area are represented

$\chi^2_3 = 2.05$, $p = 0.561$; and $\chi^2_3 = 2.05$, $p = 0.561$, respectively; *H. meridionalis* $\chi^2_1 = 1.05$, $p = 0.306$; $\chi^2_2 = 5.05$, $p = 0.080$; and $\chi^2_3 = 3.11$, $p = 0.374$, respectively). *D. galganoi* and *B. calamita* juveniles occurred in higher numbers in invaded areas, but neither season nor the invasion-by-season interaction was significant (*D. galganoi* $\chi^2_1 = 4.11$, $p = 0.043$; $\chi^2_2 = 4.19$, $p = 0.123$; $\chi^2_2 = 4.19$, $p = 0.123$, respectively; *B. calamita* $\chi^2_1 = 3.92$, $p = 0.048$ $\chi^2_2 = 5.99$, $p = 0.050$; $\chi^2_2 = 3.99$, $p = 0.136$, respectively). In the case of *P. cultripes* adults, invasion status and season were marginally significant, but their interaction was not significant ($\chi^2_1 = 3.70$, $p = 0.054$; $\chi^2_3 = 7.70$, $p = 0.053$; and $\chi^2_3 = 6.83$, $p = 0.078$, respectively). *P. cultripes* was more abundant in invaded areas than in uninvaded areas and in the fall than in the winter (Fig. 2a). In the case of *B. calamita* adults, invasion status was significant, while season and the invasion-by-season interaction were not significant ($\chi^2_1 = 5.67$, $p = 0.017$; $\chi^2_3 = 6.99$, $p = 0.072$; and $\chi^2_3 = 5.92$, $p = 0.116$, respectively). Fewer adults of *B. calamita* were observed in invaded areas (Fig. 2a).

Prey availability

A total of 5319 non-ant invertebrates and 22,386 ants (mostly Argentine ants) were captured in invaded areas. In uninvaded areas, 6545 non-ant invertebrates and 4614 native ants were captured; no Argentine ants were present. Beetles and millipedes accounted for more than 40% of the available biomass across all seasons, except in the winter, when flies were more abundant than millipedes (Fig. 3a). Total available biomass was nearly significantly different across seasons but was not affected by invasion status or the invasion-by-season interaction ($\chi^2_3 = 7.48$, $p = 0.058$; $\chi^2_1 = 3.32$, $p = 0.068$; $\chi^2_3 = 6.26$, $p = 0.100$, respectively; $N = 114$).

A total of 27,000 ants were captured, of which 22,381 were Argentine ants (Online Resource 4a). In uninvaded areas, 14 ant species were found in and under trees (Fig. 3b). Only Argentine ants were found in invaded areas (except for *Temnothorax* sp., which appeared in the summer in two invaded areas), and only native ants were found in uninvaded areas. The percentage of available ant biomass differed significantly between invaded and uninvaded areas and across seasons, but the interaction between the two factors was not significant ($\chi^2_1 = 6.35$, $p = 0.012$; $\chi^2_3 = 8.34$, $p = 0.040$; and $\chi^2_3 = 6.62$, $p = 0.085$, respectively; $N = 114$). Ant biomass

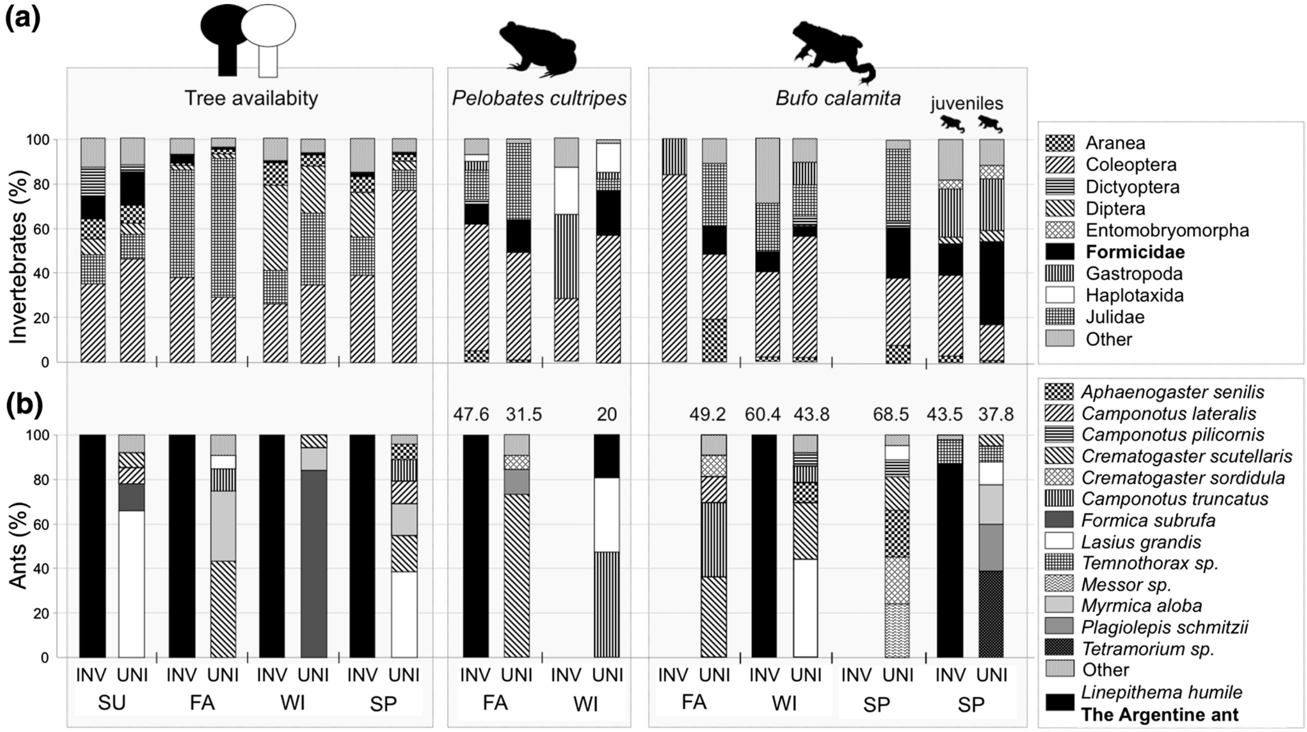


Fig. 3 Mean percentage of **a** invertebrate biomass and **b** ant species biomass (other = ant species <5% of relative ant biomass). The figures represent relative availability as estimated from pitfall traps (🌳) and relative presence in amphibian stomach contents for the different seasons in invaded (INV) versus uninvaded (UNI) areas. In **b**, the *numbers* above the *bars* indicate the percentage of ant items out of all the invertebrates found in amphibian stomachs

was greater in invaded versus uninvaded areas (Online Resource 4a).

Amphibian diet in invaded and uninvaded areas

Stomach contents were obtained from 112 amphibians (9.8% had empty stomachs, Online Resource 3a). However, isotopic samples were obtained from 106 individuals because the liver samples from six individuals had deteriorated. The cumulative prey curve for the two major amphibian species, *B. calamita* (adults and juveniles) and *P. cultripes*, reached a well-defined asymptote, indicating that the sample size was sufficient to adequately describe the amphibians' diets (Online Resource 2). For *H. meridionalis* and *D. galganoi*, sample sizes were lower, but the results are nonetheless provided for the sake of comparison.

Based on their stomach contents, the amphibians had varied diets; they consumed nine different taxonomic groups (Fig. 3a). In terms of total biomass, Coleoptera was by far the amphibians' most frequent prey (Fig. 3a); they made up 16–84% of their diets (except in the case of *D. galganoi*). Indeed Formicidae was the second or third most frequently consumed group (up to 37% of dietary biomass). Total consumed biomass did not differ based on invasion status, species, or season ($\chi^2_1 = 0.01$, $p = 0.933$; $\chi^2_3 = 2.09$, $p = 0.555$; and $\chi^2_3 = 5.23$, $p = 0.156$, respectively; $N = 95$).

Ants were found in almost all the stomachs of *B. calamita* adults and juveniles and *H. meridionalis* adults (32/34, 30/30, and 9/12, respectively; Online Resource 3a). Formicidae was less common in *P. cultripes* stomachs (both in terms of biomass and frequency). Ant frequency, mean percentage of consumed ant biomass, and the number of ant species consumed were greater for *B. calamita* (adults and juveniles) than for other species (Fig. 3a, Online Resource 4b). Thus, of the amphibians studied, *B. calamita* showed the greatest degree of ant specialization. Thirteen species of Formicidae were observed in the stomach contents: 12 native species and the Argentine ant (Online Resource 4b). Except in one individual, Argentine ants were the only ant species found in adult amphibians from invaded areas. Conversely, except in one individual, Argentine ants were completely absent from the stomachs of amphibians from uninvaded areas (Fig. 3b). Invasion status did have a significant effect on the percentage of ant biomass consumed ($\chi^2_1 = 5.04$, $p = 0.025$, $N = 95$). Significantly more ant biomass was consumed in uninvaded areas than in invaded areas (8.15 ± 2.3 versus 0.86 ± 0.2 g, respectively). Season and species did not have an effect ($\chi^2_3 = 1.87$, $p = 0.600$, and $\chi^2_3 = 1.98$, $p = 0.577$, respectively; $N = 95$).

The nitrogen isotopic baseline was significantly different across seasons and individual tree areas ($\chi^2_3 = 25.43$, $p < 0.001$ and $\chi^2_9 = 18.11$, $p = 0.034$, respectively; $N = 362$, Online Resource 3b). This finding meant that the amphibians' nitrogen isotopic values needed to be corrected. Amphibian nitrogen isotopic ratios did not differ between invaded and uninvaded areas, among species, or across seasons (invasion status $\chi^2_1 = 3.10$, $p = 0.078$; amphibian species $\chi^2_5 = 7.52$, $p = 0.185$; season $\chi^2_3 = 4.44$, $p = 0.218$; $N = 106$); the interaction between invasion status and species was not significant ($\chi^2_4 = 3.05$, $p = 0.549$, $N = 106$) (Fig. 2b). The values were highly variable, which probably explains why no effect of invasion status was found.

Preference tests

Similar results were obtained from the two types of preference tests (providing the adult toad with four ant species simultaneously or each ant species separately). *B. calamita* adults ate both native ants and Argentine ants. However, they ate native ants faster and in greater quantities (Fig. 4a). When the amphibians were simultaneously offered the four ant species, there were ant-species-specific differences in consumption ($\chi^2_3 = 406.34$, $p < 0.0001$, $N = 1400$). Fewer Argentine ants were eaten: at 30 min, around 50% of Argentine ants were left versus fewer than 30% of native ants (Fig. 4a). When we compared survivorship, the Argentine ant survived longer than the native ants: 2.03 times longer than *C. scutellaris*, 5.17 times longer than *A. senilis*, and 5.42 times longer than *T. nigerrimum*. When the amphibians were offered one ant species at a time, there were again ant-species-specific differences in consumption ($\chi^2_3 = 146.72$, $p < 0.0001$, $N = 1120$): 30% of Argentine ants remained at 30 min versus less than 20% of native ants. Once again, the Argentine ant survived longer than the native ants: 2.31 times longer than *C. scutellaris*, 2.59 times longer than *T. nigerrimum*, and 2.78 times longer than *A. senilis*. Furthermore, in the second test, no ants were eaten in six of the trials; the percentage of trials in which no ants were eaten was 21.4% for the Argentine ant (3 trials), 14.3% for *T. nigerrimum* (2 trials), 7.1% for *C. scutellaris* (1 trial), and 0% for *A. senilis*.

Amphibians from invaded versus uninvaded areas did not differ in their rates of Argentine ant consumption ($\chi^2_1 = 6\text{e}{-}04$, $p = 0.981$, $N = 1400$ observations, Fig. 4b). Both trial order and the ant-species-by-trial-order interaction were significant, meaning that there was a learning process and a significant difference among ant species in the relative degree of learning ($\chi^2_1 = 37.81$, $p < 0.0001$; $\chi^2_3 = 12.831$, $p = 0.005$, respectively, $N = 1400$ observations). Toads fed on each ant species faster in subsequent trials (*L. humile* $\chi^2_1 = 22.94$, $p < 0.001$, Fig. 4c; *A. senilis* $\chi^2_1 = 34.06$, $p < 0.001$; *T. nigerrimum* $\chi^2_1 = 20.10$, $p < 0.001$; *C. scutellaris* $\chi^2_1 = 28.82$, $p < 0.001$; $N = 350$).

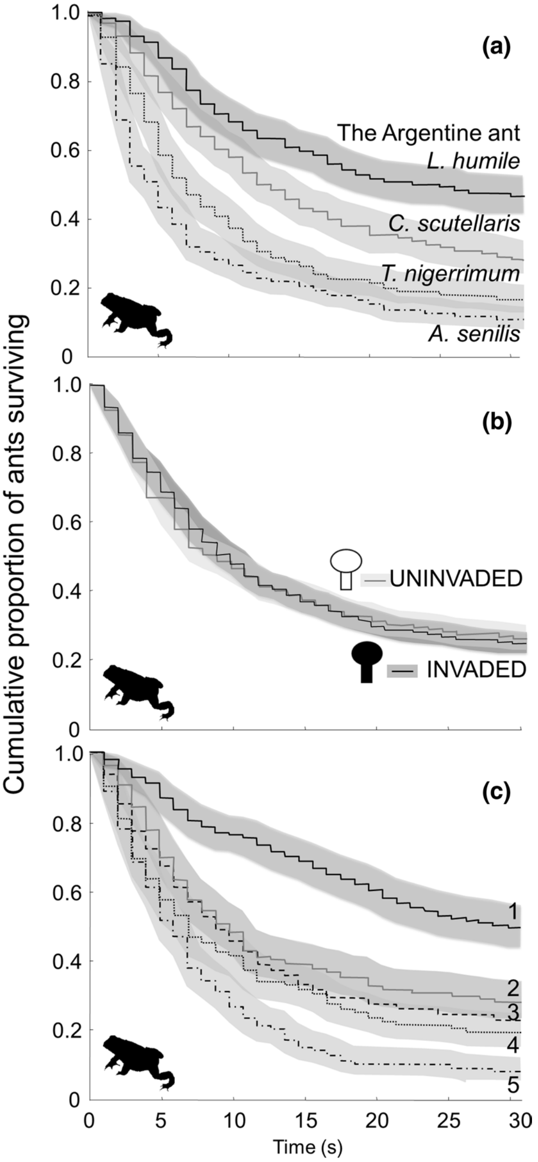


Fig. 4 Ant preferences demonstrated by *Bufo calamita*. Consumption of live ants over the course of the first trial (simultaneous exposure to four ant species): **a** by each ant species; **b** for Argentine ants exposed to *B. calamita* adults from invaded versus uninvaded areas; and **c** by trial order (1st to 5th; all ants). *Shaded areas* represent 95% CI

Discussion

Although none of the terrestrial amphibians in Doñana National Park exclusively consume ant, ants do constitute a significant percentage of their diets. Indeed, when we considered the relative representation of ants in amphibian diets, as compared to other invertebrate taxa, we found that amphibians in invaded areas consumed less ant biomass than amphibians in uninvaded areas, even though more ant biomass was available in invaded areas (but comprised only Argentine ants). These differences in consumption could be the result of a preference for native ants over Argentine ants (even post exposure). Because levels of available and consumed invertebrate biomass were similar between invaded and uninvaded areas and amphibians in invaded areas consumed less ant biomass, amphibians shifted to non-ant prey in invaded areas. The Argentine ant invasion also seems to have differentially affected the abundances of adult amphibians. While *H. meridionalis* and *D. galganoi* appeared to be unaffected, *P. cultripes* was more common in invaded areas, although this difference was less pronounced in the winter than in the fall. In contrast, *B. calamita*, the greatest ant specialist in the amphibian community, seemed to avoid invaded areas.

Effects on the amphibian community

The Argentine ant is already established in some suitable habitats in Doñana, where it has replaced most native ant species by competition (Carpintero et al. 2005, 2007; Angulo et al. 2011). According to the best known hypotheses that examine the potential relationships between invasive prey and native predators (Callaway and Ridenour 2004; Catford et al. 2009; Carlsson et al. 2009; Sax et al. 2007; Ricciardi et al. 2013), native predators are more likely to be negatively impacted if they display greater dietary specialization. Our stable isotopic analyses confirm that Doñana's terrestrial amphibian community displayed less dietary specialization than expected: δ^{15}N liver tissue values were highly variable even though the nitrogen isotopic baseline was relatively stable (Post 2002; Vanderklift and Ponsard 2003). However, ants may nonetheless make up a significant percentage of their diets (the percentage of consumed ant biomass was much greater than the percentage of available ant biomass).

To date, Argentine ant invasions had only been found to reduce predator abundance in the case of the coastal horned lizard, *P. coronatum*, a highly specialized predator of ants (Suarez et al. 2000; Fisher et al. 2002). In contrast, other ant specialists seem to have benefitted from the abundant food that stems from Argentine ant invasions (Touyama et al. 2008; Glenn and Holway 2008). In this study, we found differential effects of the invasion on adult amphibian abundance, which could be related to the species' degree of dietary specialization. The amphibian that consumed the smallest percentage of ants, *P. cultripes*, has the highest abundance of adults in invaded areas. However, there were no differences in adult abundance between invaded and uninvaded areas for *H. meridionalis* and *D. galganoi*, species that consumed intermediate percentages of ants. In contrast, we discovered that *B. calamita* adults were less abundant in invaded areas. This finding makes sense, given that *B. calamita* (as well as other bufonids; Isacch and Barg 2002) is the greatest ant specialist of the four amphibian species studied. Furthermore, in our study, the number of ants consumed, the mean percentage of ant biomass consumed, and the number of ant species consumed were greatest for *B. calamita*. However, *B. calamita* juveniles occurred in higher numbers in invaded areas than in uninvaded areas. Similar results were seen for *G. galganoi* juveniles. This contrast between adults and juveniles could be explained by the fact that juveniles are unable to choose the ponds from which they emerge, while adults can choose where they forage and breed. The greater abundance of *P. cultripes* adults in invaded areas was counterintuitive. It might be that they were attracted by the greater ant biomass in those areas or by a lower degree of interspecific competition, as other amphibian species seemed to avoid these areas (i.e., *B. calamita* adults). Although these results should be interpreted with caution because of our low amphibian sample sizes and given the difficulties associated with estimating amphibian abundance (i.e., terrestrial amphibians strongly respond to fluctuations in precipitation), we propose that Argentine ant invasions may have an effect on the most ant-specialized amphibian species.

Dietary shifts in the presence of the Argentine ant

Predators may not consume invasive prey if they are naïve, if they are absolute specialists (as per Catford et al. 2009), or if invasive prey release toxins. As a consequence, dietary shifts and reductions in prey availability occur, which are some of the mechanisms that explain the negative effects invasive prey species have on predators (Suarez et al. 2000; Suarez and Case 2002; Caut et al. 2008). In less extreme scenarios, predators should consume large quantities of the invasive prey, at least according to the exotic prey naïveté or increased susceptibility hypotheses; such may also be the case if the predator is an absolute generalist (Colautti et al. 2004; Catford et al. 2009; Li et al. 2011; Wanger et al. 2011). The amphibian community we studied here seems to provide an example of a less extreme scenario, as Argentine ants were consumed to some degree. However, the diets of Doñana amphibians clearly reflect the previously described (Angulo et al. 2011) negative effects of the Argentine ant on native ant communities: amphibians from uninvaded areas consumed a greater diversity of native ants, which largely corresponded to species availability. In contrast, the Argentine ant was almost the only ant species found in the stomach contents of amphibians from invaded areas, which also corresponded to species availability. Even if certain amphibians, such as *B. calamita* adults, seemed to avoid invaded areas, individuals may remain in them long enough to consume an entire meal of Argentine ants. Adult amphibians can have large foraging areas (Miaud et al. 2000), but we only observed two cases in which individuals ate ants that did not correspond to the area in which they were captured.

Previous work at our study site has shown that amphibians include the Argentine ant in their diets (Díaz-Paniagua et al. 2005). Indeed, the Argentine ant appears to be consumed by most ant predators, including amphibians (Ito et al. 2009), jumping spiders (Touyama et al. 2008), and pit-building ant lions (Glenn and Holway 2008). In nature, the coastal horned lizard does not consume the Argentine ant and compensates for the elimination of its main prey species (native ants) by consuming greater quantities of other invertebrates (Suarez et al. 2000; Suarez and Case 2002). In our study, we found that amphibians consumed significantly smaller percentages of ant biomass in invaded areas than in uninvaded areas. Clearly, amphibians do not completely replace native ants by Argentine ants, even when levels of Argentine ant biomass are higher than those of native ants. Doñana amphibians compensated for the lack of native ants by shifting their diet to include other invertebrates: the total biomass consumed was similar in invaded and uninvaded areas. Because the percentage of ants consumed was lower in invaded areas, the percentage of other invertebrates consumed should be higher.

Amphibian prey preferences

When native predators are faced with novel prey, they may fail to recognize or capture the introduced prey species or may be unable to consume it because it is unpalatable or contains toxins. We found that *B. calamita* adults recognized Argentine ants as prey, capturing and consuming them, albeit at markedly lower rates than for native ants. This result could stem from lower detection probabilities or lesser palatability resulting from the Argentine ant's small size or color, as seen in the case of the coastal horned lizard (Suarez et al. 2000). Of the ants tested in the laboratory, the Argentine ant was the smallest, followed by *T. nigerrimum* and *C. scutellaris*; *A. senilis* was the largest. *A. senilis* and *T. nigerrimum* are black, *C. scutellaris* is two toned (white and red), and the Argentine ant is sand colored. Thus, although the Argentine ant is most similar to *T. nigerrimum*, their survivorship patterns in the preference tests differed dramatically. The Argentine ant's marked dissimilarity in size and color might explain its higher survival rates in the laboratory experiment. In the field, of the 12 native ant species consumed by Doñana amphibians, only one (*Plagiolepis schmitzii*) is smaller than the Argentine ant (Arnan et al. 2014). Although prey movement is required to trigger feeding responses in some anurans (Oliver 1955), Doñana amphibians consumed native ants that moved faster (*A. senilis*) and slower (*C. scutellaris* and *Temnothorax* sp.) than the Argentine ant, which suggests that movement does not play a significant role. The preference for native ants could be explained by the Argentine ant having a lower energetic value. However, Pekár and Mayntz (2014) recently showed that differences in the nutritional composition of European ants cannot fully explain the preferences of predators. Finally, even if the Argentine ant has antipredatory defenses, such as aggressive behavior or noxious chemicals (Suarez and Case 2002; Glenn and Holway 2008; Robbins et al. 2013), they did not alter the response of *B. calamita* toads, which increased their feeding response with greater exposure. It could be that learning is occurring. Robbins et al. (2013) also showed that fence lizards learned to eat invasive ants over successive feeding trials. However, in our study, adults of *B. calamita* from invaded and uninvaded areas consumed Argentine ants at similar rates, indicating that prior exposure neither positively nor negatively influenced consumption.

In conclusion, when it comes to interactions between native predators and invasive prey, it is essential to consider both the direct and indirect effects of invaders on the native predator community, which means examining predator diets, prey availability, and predator feeding capacities (e.g., prey preferences, ability to learn). Although many generalist predators include ants in their diets, detailed studies on how predators are affected by Argentine ant invasions are very limited. Research on such bottom–up effects is important if we are to understand the impact of ecologically important invaders at higher trophic levels.

Acknowledgements We thanks R. Arribas, O. Blight, E. Guirlet, N. Guirlet, and P. Serpe for their help with sampling and C. Díaz-Paniagua, I. Gómez-Mestre and R. Boulay for their scientific input.

Author contribution statement EA, XC and SC conceived the ideas. SC and EA collected the data in the field and SC prepared the samples for isotopic analyses. PA-B and EA conducted the laboratory experiments and analyzed the data. PA-B led the writing of the manuscript and all the authors revised it.

Compliance with ethical standards

Funding This study was funded by the Consolider MONTES project (CSD 2008-00040); the Spanish Ministry of Economy and Competitiveness and FEDER (CGL2012-36181, CGL2013-43660-P); and fellowships to P.A.-B. (FPI program, CGL2012-36181), to S.C. (the Juan de la Cierva) and E.A. (Ramón y Cajal).

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional and national guidelines for the care and use of animals were followed.

References

Angulo E, Boulay R, Rodrigo A, Retana J, Cerda X (2007) Efecto de una especie invasora, *Linepithema humile*, la hormiga argentina, sobre la biodiversidad del Parque Nacional de Doñana (Huelva): descripción de las interacciones con las hormigas nativas. In: Ramírez L, Asensio B (eds) Investigación en Parques Nacionales: 2003–2006. Ministerio de Medio Ambiente, Madrid, pp 161–179

Angulo E, Caut S, Cerdá X (2011) Scavenging in Mediterranean ecosystems: effect of the invasive Argentine ant. Biol Invasions 13:1183–1194

Arnan X, Cerdá X, Retana J (2014) Ant functional responses along environmental gradients. J Anim Ecol 83:1398–1408

Banks PB, Dickman CR (2007) Alien predation and the effects of multiple levels of prey naiveté. Trends Ecol Evolut 22(5):229–230

Bytheway JP, Price CJ, Banks PB (2016) Deadly intentions: naïve introduced foxes show rapid attraction to odour cues of an unfamiliar native prey. Sci Rep 6:30078

Cabrera-Guzmán E, Crossland MR, Shine R (2012) Predation on the eggs and larvae of invasive cane toads (*Rhinella marina*) by native aquatic invertebrates in tropical Australia. Biol Conserv 153:1–9

Callaway RM, Ridenour WM (2004) Novel weapons: invasive success and the evolution of increased competitive ability. Front Ecol Environ 2(8):436–443

Carlsson NO, Sarnelle O, Strayer DL (2009) Native predators and exotic prey—an acquired taste? Front Ecol Environ 7:525–532

Carpintero S, Reyes-López J, Arias de Reyna L (2005) Impact of Argentine ants (*Linepithema humile*) on an arboreal ant community in Doñana National Park, Spain. Biodivers Conserv 14:151–163

Carpintero S, Retana J, Cerdá X, Reyes-López J, Arias de Reyna L (2007) Exploitative strategies of the invasive Argentine ant (*Linepithema humile*) and native ant species in a southern Spanish pine forest. Environ Entomol 36:1100–1111

Carthey AJ, Banks PB (2014) Naïveté in novel ecological interactions: lessons from theory and experimental evidence. Biol Rev 89(4):932–949

Catford JA, Jansson R, Nilsson C (2009) Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. Divers Distrib 15(1):22–40

Caut S, Angulo E, Courchamp F (2008) Dietary shift of an invasive predator: rats, seabirds and sea turtles. J Appl Ecol 45:428–437

Caut S, Angulo E, Courchamp F (2009) Variation in discrimination factors (Δ15N and Δ13C): the effect of diet isotopic values and applications for diet reconstruction. J Appl Ecol 46(2):443–453

Colautti RI, Ricciardi A, Grigorovich IA, MacIsaac HJ (2004) Is invasion success explained by the enemy release hypothesis? Ecol Lett 7:721–733

Díaz-Paniagua C, Gómez-Rogríguez C, Portheault, de Vries, W (2005) Los anfibios de Doñana. Organismo Autónomo De Parques Nacionales, Ministerio de Medio Ambiente, Estación Biológica de Doñana, Spain

Díaz-Paniagua C, Fernández-Zamudio R, Florencio M, García-Murillo P, Gómez-Rodríguez C, Portheault A, Serrano L, Siljeström P (2010) Temporay ponds from Doñana National Park: a system of natural habitats for the preservation of aquatic flora and fauna. Limnetica 29(1):41–58

Estany-Tigerström D, Bas JM, Pons P (2010) Does Argentine ant invasion affect prey availability for foliage-gleaning birds? Biol Invasions 12:827–839

Estany-Tigerström D, Bas JM, Clavero M, Pons P (2013) Is the blue tit falling into an ecological trap in Argentine ant invaded forests? Biol Invasions 15:2013–2027

Fisher RN, Suarez AV, Case TJ (2002) Spatial patterns in the abundance of the coastal horned lizard. Conserv Biol 16:205–215

Glenn S, Holway D (2008) Consumption of introduced prey by native predators: Argentine ants and pit-building ant lions. Biol Invasions 10:273–280

Gordon DM, Heller NE (2014) The invasive Argentine ant *Linepithema humile* (Hymenoptera: Formicidae) in Northern California reserves: from foraging behavior to local spread. Mirmecol News 19:103–110

Gove AD, Majer JD, Rico-Gray V (2009) Ant assemblages in isolated trees are more sensitive to species loss and replacement than their woodland counterparts. Basic Appl Ecol 10(2):187–195

Heller NE, Ingram KK, Gordon DM (2008) Nest connectivity and colony structure in unicolonial Argentine ants. Insect Soc 55(4):397–403. doi:10.1007/s00040-008-1019-0

Hoffmann M, Hilton-Taylor C, Angulo A, Böhm M, Brooks TM, Butchart SH et al (2010) The impact of conservation on the status of the world's vertebrates. Science 330:1503–1509

Holway DA, Suarez AV (2006) Homogenization of ant communities in mediterranean California: the effects of urbanization and invasion. Biol Conserv 127(3):319–326

Holway DA, Lach L, Suarez AV, Ysutsui ND, Case TJ (2002) The causes and consequences of ant invasions. Annu Rev Ecol Syst 33:181–233

Hooper-Bui LM, Rust MK, Reierson DA (2004) Predation of the endangered California Least Tern*, Sterna antillarum browni* by the Southern Fire Ant, *Solenopsis xyloni* (Hymenoptera, Formicidae). Sociobiology 43:401–418

Isacch JP, Barg M (2002) Are bufonid toads specialized ant-feeders? A case test from the Argentinian flooding pampa. J Nat Hist 36:2005–2012

Ito F, Okaue M, Ichikawa T (2009) A note on prey composition of the Japanese treefrog, Hyla japonica, in an area invaded by Argentine ants, *Linepithema humile*, in Hiroshima Prefecture, western Japan (Hymenoptera: Formicidae). Myrmecol News 12:35–39

Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. Trends Ecol Evol 17:164–170

Kurz-Benson K, Otieno D, Lobo do Vale R, Siegwolf R, Schmidt M, Herd A et al (2006) Hydraulic lift in cork oak trees in a savannah-type Mediterranean ecosystem and its contribution to the local water balance. Plant Soil 282:361–378

Lach L, Parr C, Abbott K (2010) Ant ecology. Oxford University Press, Oxford

Li Y, Ke Z, Wang S, Smith GR, Liu X (2011) An exotic species is the favorite prey of a native enemy. PLoS One 6(9):e24299

Lorrain A, Graham BS, Popp BN, Allain V, Olson RJ, Hunt BPV, Potier M, Fry B, Galván-Magaña F, Menkes CER, Kaehler S, Ménard F (2014) Nitrogen isotopic baselines and implications for estimating foraging habitat and trophic position of yellowfin tuna in the Indian and Pacific Oceans. Deep-Sea Res Pt II 113:188–198

Lowe S, Browne M, Boudjelas S, De Poorter M (2000) 100 of the world's worst invasive alien species: a selection from the global invasive species database. Invasive Species Specialist Group Auckland, New Zealand

Luque GM, Bellard C, Bertelsmeier C, Bonnaud E, Genovesi P, Simberloff D, Courchamp F (2013) The 100th among some of the worst. Biol Invasions 16:981–985

Maerz JC, Karuzas JM, Madison DM, Blossey B (2005) Introduced invertebrates are important prey for a generalist predator. Divers Distrib 11:83–90

Miaud C, Sanuy D, Avrillier JN (2000) Terrestrial movements of the natterjack toad *Bufo calamita* (Amphibia, Anura) in a semi-arid, agricultural landscape. Amphibia Reptilia 21:357–370

Monzo C, Juan-Blasco M, Pekar S, Molla O, Castanera P, Urbaneja A (2013) Pre-adaptive shift of a native predator (Araneae, Zodariidae) to an abundant invasive ant species (Hymenoptera, Formicidae). Biol Invasions 15:89–100

Oliver JA (1955) The natural history of North American amphibians and reptiles. Princeton, New Jersey

Pekár S, Mayntz D (2014) Comparative analysis of the macronutrient content of Central European ants (Formicidae): implications for ant-eating predators. J Insect Physiol 62:32–38

Pintor LM, Byers JE (2015) Do native predators benefit from non-native prey? Ecol Lett 18:1174–1180

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83(3):703–718

Pysek P, Richardson DM, Pergl J, Jarosik V, Sixtová Z, Weber E (2008) Geographical and taxonomic biases in invasion ecology. Trends Ecol Evol 23(5):237–244

R Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/. Accessed 16 Aug 2017

Ricciardi A, Hoopes MF, Marchetti MP, Lockwood JL (2013) Progress toward understanding the ecological impacts of nonnative species. Ecol Monogr 83:263–282

Robbins TR, Freidenfelds NA, Langkilde T (2013) Native predator eats invasive toxic prey: evidence for increased incidence of consumption rather than aversion-learning. Biol Invasions 15:407–415

SAS Institute Inc® (2008) 9.2 User Guide. SAS Inst, Cary, NC, USA

Sax DF, Stachowicz JJ, Brown JH, Bruno JF, Dawson MN, Gaines SD, Grosberg RK, Hastings A, Holt RD, Mayfield MM, O'Connor MI, Rice WR (2007) Ecological and evolutionary insights from species invasions. Trends Ecol Evol 22(9):465–471

Sih A, Bolnick DI, Luttbeg B, Orrock JL, Peacor SD, Pintor LM, Preisser E, Rehage JS, Vonesh JR (2010) Predator–prey naïveté, antipredator behavior, and the ecology of predator invasions. Oikos 119:610–621

Sockman KW (1997) Variation in life-history traits and nest-site selection affects risk of nest predation in the California gnatcatcher. Auk 114:324–332

Suarez AV, Case TJ (2002) Bottom-up effects on persistence of a specialist predator: ant invasions and horned lizards. Ecol Appl 12:291–298

Suarez AV, Bolger DT, Case TJ (1998) Effects of fragmentation and invasion on native ant communities in coastal southern California. Ecology 79(6):2041–2056

Suarez AV, Richmond JQ, Case TJ (2000) Prey selection in horned lizards following the invasion of Argentine ants in southern California. Ecol Appl 10:711–725

Suarez AV, Holway DA, Case TJ (2001) Patterns of spread in biological invasions dominated by long-distance jump dispersal: insights from Argentine ants. P Natl Acad Sci USA 98:1095–1100

Suarez A, Yeh P, Case TJ (2005) Impacts of Argentine ants on avian nesting success. Insect Soc 52:378–382

Therneau TM (2015) coxme: Mixed Effects Cox Models. R package version 2.2-5. https://CRAN.R-project.org/package=coxme. Accessed 16 Aug 2017

Touyama Y, Ihara Y, Ito F (2008) Argentine ant infestation affects the abundance of the native myrmecophagic jumping spider *Siler cupreus* Simon in Japan. Insect Soc 55:144–146

Twardochleb LA, Novak M, Moore JW (2012) Using the functional response of a consumer to predict biotic resistance to invasive prey. Ecol Appl 22(4):1162–1171

Vanderklift MA, Ponsard S (2003) Sources of variation in consumer-diet δN-15 enrichment: a meta-analysis. Oecologia 136(2):169–182

Vogel V, Pedersen JS, Giraud T, Krieger MJ, Keller L (2010) The worldwide expansion of the Argentine ant. Divers Distrib 16(1):170–186

Wanger TC, Wielgoss AC, Motzke I, Clough Y, Brook BW, Sodhi NS, Tscharntke T (2011) Endemic predators, invasive prey and native diversity. P Roy Soc B-Biol Sci 278:690–694

Wetterer JK, Wild AL, Suarez AV, Roura-Pascual N, Espadaler X (2009) Worldwide spread of the Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae). Mirmecol News 12:187–194

List of Tables

List of Figures

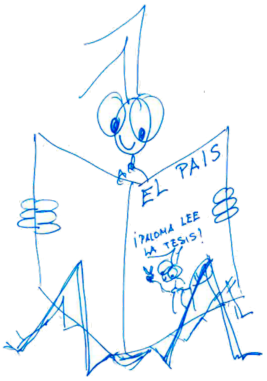
List of Figures (*continued*)

Funding

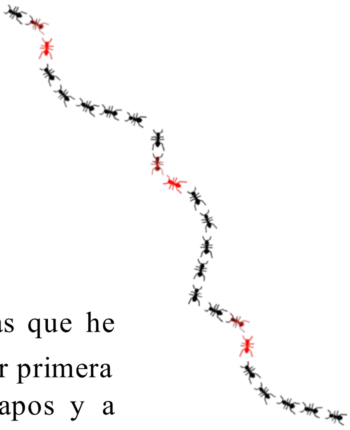
I was granted with a Predoctoral fellowship FPI program (BES-2013-064713) of the Spanish Ministry of Economy and Competitiveness, which also financed my short-stay at Tel Aviv University in Israel (EEBB-I-15-09870), another at the Université François Rabelais in France (EEBB-I-16-10609) and a third one at the Universidad de Montevideo in Uruguay (EEBB-I-17-12165). In addition, I also obtained each year, from 2014 to 2017 the EDUPO grant from the Universidad Pablo de Olavide (EDUPO program, Ayudas destinadas a cubrir gastos para la mejora cualitativa en el desarrollo de tesis doctorales) to attend congresses or cover the English editing to publish my research. The work of my thesis was funded by the Spanish MINECO and FEDER (Consolider MONTES CSD2008-00040, CGL2012-36181 and CGL2015-65807-P to XC, and CGL2013-43660-P to EA). Additional funding came from the EBD (MINECO Severo Ochoa Program for Centers of Excellence in R+D+I [SEV-2012-0262] to EA).

Agradecimientos / Acknowledgements / Remerciements

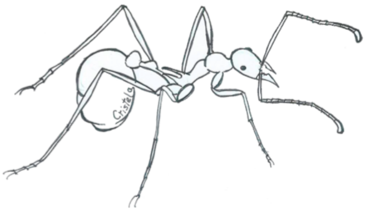
Esta aventura llamada tesis no habría sido posible sin la ayuda de personas a las que quiero agradecer profundamente su apoyo. En primer lugar a mis directores, Elena y Xim. Por haber confiado en mí para embarcarme en este gran proyecto, por vuestro apoyo e implicación desde el primer al último momento, por vuestra paciencia, por todo lo que me habéis enseñado. A Xim, por tu sensatez, sencillez y sinceridad. Por sacar siempre hueco a pesar de los pesares, por sacrificar rondas de cervezas, por tu cercanía y tus acertados consejos (al final, siempre tengo que darte la razón). A Elena, mi mentora, por haberme guiado en este camino. Tienes mi admiración como científica y como persona, aunque insistas en que no te use como ejemplo, para mí lo eres. Eres un ejemplo de esfuerzo y tesón, ingenio y eficiencia, compañerismo, energía y empatía, todo ello necesario para hacer buena ciencia. Gracias por el enorme el esfuerzo que has volcado conmigo en esta tesis, no tiene precio todo lo que me has enseñado y lo mucho que me has apoyado. Has sido para mí mucho más que una directora de tesis, gracias.



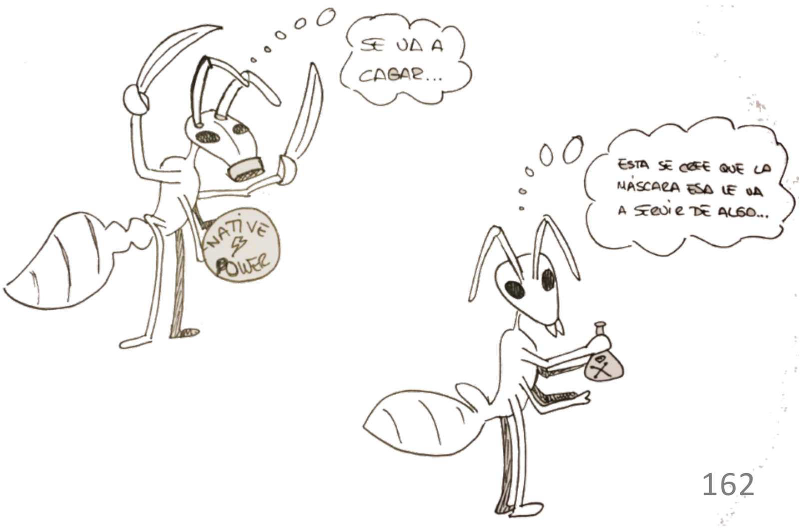
I would like to thank the work of all co-authors (other than my supervisors) that have collaborated in each Section of this thesis: *Section 1*: Stephane Caut (EBD – CSIC); *Section 2*: Abraham Hefetz (Tel Aviv University), Raphaël Boulay and Alain Lenoir (IRBI, Université F. Rabelais de Tours), Alejandro Bertó-Moran, Carmen Díaz-Paniagua and H. Christoph Liedtke (EBD – CSIC), Johan Billen (Katholieke Universiteit Leuven), Kamlesh R. Chauhan and Ganga Bhagavathy (USDA Agricultural Research Center); *Section 3*: Raphaël Boulay (IRBI – CNRS), Iván Gómez-Mestre and Alejandro Bertó-Moran and Ana Carvajal (EBD – CSIC); *Section 4*: Juli Broggi and Oscar González-Jarri (EBD – CSIC).

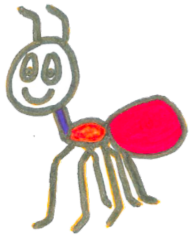


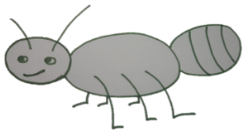
Gracias a todas las personas que me han guiado por Doñana, con las que he disfrutado del campo y de la vida en El Palacio. A Poli, por mostrarme por primera vez el sonido de las noches de Doñana, por enseñarme a capturar sapos y a cuidarlos. Eres una gran herpetóloga, luchadora incansable por la conservación de la biodiversidad y mejor persona. A Kiko, Rosita, María León, Miguel y Gema, Vane y Miguel, Paqui, Elisa, Julie, Rocío, Ale y Fabrizio, Pilar y Fernando, cocineras y guardas, y a todos aquellos con los que ir a Doñana era, si cabe, aún mejor. A todos los amigos (*voluntarios*) que me habéis acompañado en las revisiones de *pajaritos* y en el trabajo de campo. La que sin duda se lleva la palma es mi amiga María, siempre dispuesta a madrugar para ir al campito conmigo, hacer la maratón y volver a tiempo de llegar a la piscina. Gracias por eso y por mucho más. A Benja, pour ta aide sur le terrain et dans le labo, pour votre grand enthousiasme. A Óscar, por tu impecable trabajo, por procurarles tanto mimo a los pollitos, por enseñarme sobre carboneros, sobre ciencia y sobre la vida, por los enriquecedores debates y por tus buenos consejos.



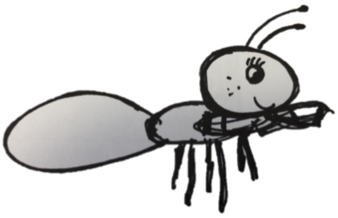
A los miembros de mi comité de tesis, por aguantar mis presentaciones eternas y además aportar un debate productivo y buenas ideas: Iván, Raphaël, Pepe Tella y Joaquín Reyes. Al comité de experimentación animal, por facilitar el arduo procedimiento. A todos y cada uno de los animales que habéis participado en este estudio, sapos, carboneros y hormigas, aunque estas últimas no sean, todavía, animales. A Alex Bertó, por tu inestimable ayuda con los experimentos de los sapitos. A Juli, por tu ayuda en el campo, tus correcciones y tu sinceridad. Merci à Olivier, pour t'aide et conseil au début de la thèse, pour ta sympathie. A Marce por recibirme con los brazos abiertos en Sevilla y guiarme en mis inicios en el mundo *ebediano*.



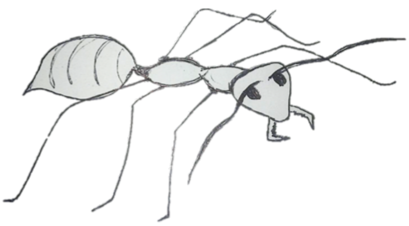




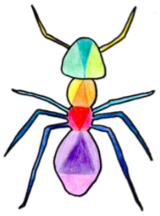
A los investigadores que me habéis acogido en sus respectivos países, *a levante y a poniente*: Hefetz (Israel), Raphaël (Francia), Raúl Maneyro (Uruguay), Roxy (Argentina). Gracias por facilitarme la estancia, por vuestra implicación y cercanía. Por enseñarme técnicas de laboratorio, análisis estadísticos, ecología y mucho más. Gracias también a todas las personas que habéis hecho de mis estancias una grata experiencia. A Neta, to Tovit and her family, to my collegues in the lab at the Zoo, with the best view, à Alain, Cristela e Isa, a Feline y David, a Juan y Lucía, y a los demás *gurises*. Gracias a Raphaël, Irene y Anaïs, por darme cobijo en vuestro hogar y laboratorio, por hacerme sentir como en casa y por vuestra calidez. Rapha, gracias por esa chispa de ironía que a mí me encantaba, por tu dedicación, cercanía y consejo. Has sido un mentor para mí, un beso al viento.

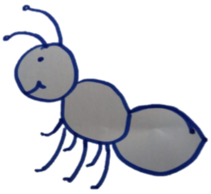


Al *grupo hormigas*. A Ana Carvajal por ser tan resolutiva en todo lo que te propones, por tu ayuda en el día a día y por ese arte coriano que no se *pué aguantá*. A Fernando Amor, por tus consejos e ideas, por transmitir como nadie conocimiento, por ser tan entrañable. A Juanma, a Basti y a Mamen, por vuestra gran ayuda como técnicos y por vuestra alegría sureña. Al mejor despacho de la EBD, no solo por ser un buen área de forrajeo, si no por cada una de las personas que por allí habéis pasado durante mi estancia: Sara, Edu, Cristela, Sofrita, J, Sar, David, Olivier, Benja, Ana *la portuguesa*, Lea, Isa *la benjamina*, Isa *la sanluqueña* y el resto de aledaños que por allí pasabais a saludar, consultar dudas y hacernos reír. Cada uno de vosotros ha ido dejando huella, no sólo en el *facedoor*, si no en mi interior. Gracias por las sonrisas, las carcajadas, las ayudas con los quebraderos de cabeza de la estadística, con los scripts, las recogidas de libros de la biblioteca, los ánimos, los desahogos, los continuos chistes, los abrazos y un sin fin de aportaciones imposibles de enumerar. Habéis hecho que me sintiera como en casa (a veces casi que lo parecía, jaja). Ese buen rollo a diario vale oro, gracias. A los que me habéis tendido una mano (o un brazo) en los diferentes laboratorios, siempre dispuestos ayudar con una sonrisa en la cara: Nene, Olaya, Pablo Burraco y Laura, entre otros. A Isa y David por vuestra paciencia y trabajo con los mapas. A Manolo de colecciones por hacerme esa lanceta maravillosa para perforar huevos de codorniz sin romperlos, también por enseñarme tu minucioso trabajo.



Y como fuera del despacho y del *labo* también hay vida dentro de la EBD, gracias a todos aquellos que os esmeráis en el día a día por permitir que la investigación se lleve a cabo, por poner vuestro granito (o granazo) de arena en mi tesis, allanando el camino. Especial mención a Pedro, por saberte mi nombre desde el primer día que pisé la Estación y por abrirme la puerta a horas intempestivas; a Antonio Jesús López, por tu eficacia para resolver temas administrativos; a Marina, Mª Ángeles y Joaquín (y Gema, *foránea hormigoloca*), por vuestra gran labor en la búsqueda de bibliografía; y a Jesús y Luis por solucionarme los problemas de una de las herramientas de trabajo más importantes, el dichoso ordenador. No me olvido de todos los demás, personal de centralita, administración, dirección, compras, colecciones, biblioteca, limpieza, un largo etcétera tanto de la Estación, como de la Reserva.





A mis amig@s. A todas y cada una de las que no habéis dejado de apoyarme en todo momento durante este viaje. A las de cerca, mi familia sevillana, vecinas invasoras y compañeros de hogar y de aventuras. A las de lejos, que siempre habéis estado cerca, nos veremos pronto en los campos y en los montes.

A mi familia, por vuestro apoyo incansable, contra viento y marea, siempre estáis ahí. Gracias por enseñarme a valorar la naturaleza, a luchar por mis metas y a disfrutar de cada momento.

A todos y cada uno de los que me quedan en el tintero, pero sabéis de buena fe que os agradezco vuestra ayuda.

Bibliography

Abril S, Gómez C (2011) Aggressive behaviour of the two European Argentine ant supercolonies (Hymenoptera: Formicidae) towards displaced native ant species of the northeastern Iberian Peninsula. Myrmecological News 14:99-106

Abril S, Gómez López C (2009) Ascertaining key factors behind the coexistence of the native ant species *Plagiolepis pygmaea* with the invasive Argentine ant *Linepithema humile* (Hymenoptera: Formicidae). Sociobiology 53:559-568

Abril S, Oliveras J, Gomez C (2007) Foraging activity and dietary spectrum of the argentine ant (Hymenoptera: Formicidae) in invaded natural areas of the Northeast Iberian Peninsula. Environ. Entomol. 36:1166-1173

Aho T, Kuitunen M, Suhonen J, Jäntti A, Hakkari T (1997) Behavioural responses of Eurasian treecreepers, *Certhia familiaris*, to competition with ants. Animal Behaviour 54:1283-1290

Aho T, Kuitunen M, Suhonen J, Jäntti A, Hakkari T (1999) Reproductive success of Eurasian treecreepers, *Certhia familiaris*, lower in territories with wood ants. Ecology 80:998-1007

Allen C, Birge H, Slater J, Wiggers E (2017) The invasive ant, *Solenopsis invicta*, reduces herpetofauna richness and abundance. Biological Invasions:1-10

Allen C, Epperson D, Garmestani A (2004) Red imported fire ant impacts on wildlife: a decade of research. The American midland naturalist 152:88-103

Allen CR, Rice KG, Wojcik DP, Percival HF (1997) Effect of red imported fire ant envenomization on neonatal American alligators. Journal of Herpetology 31:318-321

Alvarez-Blanco P, Caut S, Cerdá X, Angulo E (2017) Native predators living in invaded areas: responses of terrestrial amphibian species to an Argentine ant invasion. Oecologia 185:95-106

AmphibiaWeb. Available from https://amphibiaweb.org Accessed 24th November 2018

Andersen AN, Majer JD (2004) Ants show the way down under: invertebrates as bioindicators in land management. Frontiers in Ecology and the Environment 2:291-298

Angulo E, Boulay R, Rodrigo A, Retana J, Cerdá X (2007) Efecto de una especie invasora, *Linepithema humile*, la hormiga argentina, sobre la biodiversidad del Parque Nacional de Doñana (Huelva): descripción de las interacciones con las hormigas nativas. In: eds. LRBA (ed) Proyectos de investigación en Parques Nacionales 2003-2006. Organismo Autónomo Parques Nacionales, Madrid, pp. 161-179

Angulo E, Boulay R, Ruano F, Tinaut A, Cerdá X (2016) Anthropogenic impacts in protected areas: assessing the efficiency of conservation efforts using Mediterranean ant communities. PeerJ 4:e2773

Angulo E, Caut S, Cerdá X (2011) Scavenging in Mediterranean ecosystems: effect of the invasive Argentine ant. Biological Invasions 13:1183-1194

AntWeb. Available from http://www.antweb.org Accessed 29th October 2018

Antwis R, Browne R (2009) Ultraviolet radiation and vitamin D3 in amphibian health, behaviour, diet and conservation. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 154:184-190

Ardia DR, Pérez JH, Clotfelter ED (2006) Nest box orientation affects internal temperature and nest site selection by tree swallows. Journal of Field Ornithology 77:339-344

Arnan X, Cerdá X, Retana J (2012) Distinctive life traits and distribution along environmental gradients of dominant and subordinate Mediterranean ant species. Oecologia 170:489-500

Arnan X, Cerdá X, Retana J (2017) Relationships among taxonomic, functional, and phylogenetic ant diversity across the biogeographic regions of Europe. Ecography 40:448-457

Attygalle AB, Morgan ED (1984) Chemicals from the glands of ants. Chemical Society Reviews 13:245-278

Banks PB, Dickman CR (2007) Alien predation and the effects of multiple levels of prey naivete. Trends Ecol Evol 22:229-30; author reply 230-1

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1-48

Bea A, Montori A, Pascual X (1994) Herpetofauna dels Aiguamolls de l'Empordà. ICHN Els sistemes naturals dels Aiguamolls de l'Empordà. Treb. lnst. Cat. Hist. Nat:13

Beard KH (2007) Diet of the invasive frog, *Eleutherodactylus coqui*, in Hawaii. Copeia 2007:281-291

Bellard C, Cassey P, Blackburn TM (2016) Alien species as a driver of recent extinctions. Biology Letters 12:20150623

Bellard C, Jeschke J (2016) A spatial mismatch between invader impacts and research publications. Conservation Biology 30:230-232

Berazategui M, Camargo A, Maneyro R (2007) Environmental and seasonal variation in the diet of *Elachistocleis bicolor* (Guérin-Méneville 1838)(Anura: Microhylidae) from northern Uruguay. Zoological Science 24:225-231

Berenbaum MR (1995) The chemistry of defense: theory and practice. Proceedings of the National Academy of Sciences 92:2-8

Bertelsmeier C, Blight O, Courchamp F (2016) Invasions of ants (Hymenoptera: Formicidae) in light of global climate change. Myrmecol. News 22:25-42

Bertelsmeier C, Ollier S, Liebhold A, Keller L (2017) Recent human history governs global ant invasion dynamics. Nat Ecol Evol 1:1-8

Bivand R, Rundel C (2017) rgeos: Interface to geometry engine-open source ('geos'). R package version 0.3-26. https://CRAN.R-project.org/package=rgeos

Blackburn TM, Cassey P, Duncan RP, Evans KL, Gaston KJ (2004) Avian extinction and mammalian introductions on oceanic islands. Science 305:1955-1958

Blackburn TM, Essl F, Evans T, Hulme PE, Jeschke JM, Kühn I, Kumschick S, Marková Z, Mrugała A, Nentwig W, Pergl J, Pysek P, Rabitsch W, Ricciardi A, Richardson DM, Sendek A, Vilà M, Wilson JR, Winter M, Genovesi P, Bacher S (2014) A unified classification of alien species based on the magnitude of their environmental impacts. PLoS biology 12:e1001850

Blight O, Berville L, Vogel V, Hefetz A, Renucci M, Orgeas J, Provost E, Keller L (2012) Variation in the level of aggression, chemical and genetic distance among three supercolonies of the Argentine ant in Europe. Molecular Ecology 21:4106-4121

Blight O, Josens R, Bertelsmeier C, Abril S, Boulay R, Cerdá X (2017) Differences in behavioural traits among native and introduced colonies of an invasive ant. Biological Invasions 19:1389-1398

Blossey B, Notzold R (1995) Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. Journal of Ecology 83:887-889

Blum MS (1996) Semiochemical parsimony in the Arthropoda. Annual review of entomology 41:353-374

Blum S, Hermann Jr H (1978) Venoms and venom apparatuses of the Formicidae: Dolichoderinae and Aneuretinae. Arthropod venoms. Springer, pp. 871-894

Boieiro M, Catry P, Jardim CS, Menezes D, Silva I, Coelho N, Oliveira P, Gatt MC, Pedro P, Granadeiro JP (2018) Invasive Argentine ants prey on Bulwer's petrels nestlings on the Desertas Islands (Madeira) but do not depress seabird breeding success. Journal for Nature Conservation

Bosch J, Fernández ‐ Beaskoetxea S, Garner TW, Carrascal LM (2018) Long ‐ term monitoring of an amphibian community after a climate change ‐ and infectious disease ‐ driven species extirpation. Global change biology 24:2622-2632

Bradley BA, Blumenthal DM, Wilcove DS, Ziska LH (2010) Predicting plant invasions in an era of global change. Trends in ecology & evolution 25:310-318

Brandt M, Van Wilgenburg E, Tsutsui ND (2009) Global-scale analyses of chemical ecology and population genetics in the invasive Argentine ant. Molecular Ecology 18:997-1005

Broggi J, Senar JC (2009) Brighter great tit parents build bigger nests. Ibis 151:588-591

Brook BW, Sodhi NS, Bradshaw CJ (2008) Synergies among extinction drivers under global change. Trends in ecology & evolution 23:453-460

Buege JA, Aust SD (1978) Microsomal lipid peroxidation. In: Fleischer S and Packer L (eds) Methods in Enzymology. Academic Press, pp. 302-310

Bytheway JP, Price CJ, Banks PB (2016) Deadly intentions: naïve introduced foxes show rapid attraction to odour cues of an unfamiliar native prey. Scientific reports 6:30078

Cabrera-Guzmán E, Crossland MR, Brown GP, Shine R (2013) Larger body size at metamorphosis enhances survival, growth and performance of young cane toads (*Rhinella marina*). PLoS One 8:e70121

Cabrera-Guzmán E, Crossland MR, Pearson D, Webb JK, Shine R (2015) Predation on invasive cane toads (*Rhinella marina*) by native Australian rodents. Journal of pest science 88:143-153

Cabrera-Guzmán E, Crossland MR, Shine R (2012) Predation on the eggs and larvae of invasive cane toads (*Rhinella marina*) by native aquatic invertebrates in tropical Australia. Biological Conservation 153:1-9

Caldwell JP (1996) The evolution of myrmecophagy and its correlates in poison frogs (Family Dendrobatidae). Journal of Zoology (London) 240(1):75-101

Callaway RM, Ridenour WM (2004) Novel weapons: invasive success and the evolution of increased competitive ability. Frontiers in Ecology and the Environment 2:436-443

Carlsson NOL, Sarnelle O, Strayer DL (2009) Native predators and exotic prey –an acquired taste? Frontiers in Ecology and the Environment 7:525-532

Carmagnol F, Sinet PM, Jerome H (1983) Selenium-dependent and non-selenium-dependent glutathione peroxidases in human tissue extracts. Biochimica et Biophysica Acta (BBA)-General Subjects 759:49-57

Carpintero S (2001) Repercusión de la hormiga argentina (*Linepithema humile*) en el Parque Nacional de Doñana. Tesis doctoral. Universidad de Córdoba. Inèdita,

Carpintero S, Retana J, Cerdá X, Reyes-López J, Arias de Reyna L (2007) Exploitative Strategies of the Invasive Argentine Ant (*Linepithema humile*) and Native Ant Species in a Southern Spanish Pine Forest. Environmental Entomology 36:1100-1111

Carpintero S, Reyes-López J (2008) The role of competitive dominance in the invasive ability of the Argentine ant (*Linepithema humile*). Biological Invasions 10:25-35

Carpintero S, Reyes-Lopez J, de Reyna LA (2003) Impact of human dwellings on the distribution of the exotic Argentine ant: a case study in the Doñana National Park, Spain. Biological Conservation 115:279-289

Carpintero S, Reyes-López J, De Reyna LA (2005) Impact of Argentine ants (*Linepithema humile*) on an arboreal ant community in Donana National Park, Spain. Biodiversity & Conservation 14:151-163

Carthey AJ, Banks PB (2014) Naïveté in novel ecological interactions: lessons from theory and experimental evidence. Biological Reviews 89:932-949

Catford JA, Jansson R, Nilsson C (2009) Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. Diversity and distributions 15:22-40

Caut S, Angulo E, Courchamp F (2008) Dietary shift of an invasive predator: rats, seabirds and sea turtles. Journal of Applied Ecology 45:428-437

Caut S, Angulo E, Courchamp F (2009) Variation in discrimination factors (Δ15N and Δ13C): the effect of diet isotopic values and applications for diet reconstruction. Journal of Applied Ecology 46:443-453

Cavill GWK, Clark DV (1971) Ant secretions and cantharidin. In: Crosby. MJaDG (ed) Naturally occurring insecticides. Marcel Dekker, New York, pp. 271-305

Cavill GWK, Houghton E, McDonald FJ, Williams PJ (1976) Isolation and characterization of dolichodial and related compounds from the Argentine ant, *Iridomyrmex humilis*. Insect Biochemistry 6:483-490

CBD, Convention on Biological Diversity. https://www.cbd.int/convention/ Accessed 9th November 2018

Ceballos G, Ehrlich PR, Barnosky AD, García A, Pringle RM, Palmer TM (2015) Accelerated modern human–induced species losses: Entering the sixth mass extinction. Science Advances 1:e1400253

Cerdá X, Angulo E, Boulay R, Lenoir A (2009) Individual and collective foraging decisions: a field study of worker recruitment in the gypsy ant *Aphaenogaster senilis*. Behavioral Ecology and Sociobiology 63(4):551-562

Cerda X, Arnan X, Retana J (2013) Is competition a significant hallmark of ant (Hymenoptera: Formicidae) ecology. Myrmecological News 18:131-147

Chauhan KR, Schmidt W (2014) Biorational synthesis of iridomyrmecin diastereomers from catnip oil. Tetrahedron Letters 55:2534-2536

Clarke GM (1995) Relationships between developmental stability and fitness: application for conservation biology. Conservation Biology 9:18-24

Clavero M (2016) Species substitutions driven by anthropogenic positive feedbacks: Spanish crayfish species as a case study. Biological Conservation 193:80-85

Clavero M, García-Berthou E (2005) Invasive species are a leading cause of animal extinctions. Trends in ecology & evolution 20:110

Clobert J, Perrins C, McCleery R, Gosler AG (1988) Survival rate in the great tit *Parus major* in relation to sex, age, and immigration status. The Journal of Animal Ecology 57:287-306

Cohen AA, McGraw KJ, Robinson WD (2009) Serum antioxidant levels in wild birds vary in relation to diet, season, life history strategy, and species. Oecologia 161:673-683

Cohen G, Somerson NL (1969) Catalase-aminotriazole method for measuring secretion of hydrogen peroxide by microorganisms. Journal of bacteriology 98:543-546

Colautti RI, Ricciardi A, Grigorovich IA, MacIsaac HJ (2004) Is invasion success explained by the enemy release hypothesis? Ecology Letters 7:721-733

Collingwood C, Yarrow I (1969) A survey of Iberian Formicidae. EOS 44:53-101

Costantini D (2014) Oxidative stress and hormesis in evolutionary ecology and physiology. A marriage between mechanistic and evolutionary approaches. Springer.

Cott HB (1934) On the ecology of *Hyla arborea* var. *meridionalis* in Gran Canaria, with special reference to predatory habits considered in relation to the protective adaptations of insects. P Zool Soc Lond 104(2):311-331

Courchamp F, Angulo E, Rivalan P, Hall RJ, Signoret L, Bull L, Meinard Y (2006) Rarity value and species extinction: the anthropogenic allee effect. PLoS biology 4:e415

Courchamp F, Langlais M, Sugihara G (2000) Rabbits killing birds: modelling the hyperpredation process. Journal of Animal Ecology 69:154-164

Cribb AE, Leeder JS, Spielberg SP (1989) Use of a microplate reader in an assay of glutathione reductase using 5, 5′ -dithiobis (2-nitrobenzoic acid). Analytical biochemistry 183:195-196

Crooks JA (2002) Characterizing ecosystem ‐ level consequences of biological invasions: the role of ecosystem engineers. Oikos 97:153-166

Crooks JA (2005) Lag times and exotic species: The ecology and management of biological invasions in slow-motion1. Ecoscience 12:316-329

Crossland MR (2000) Direct and indirect effects of the introduced toad *Bufo marinus* (Anura: Bufonidae) on populations of native anuran larvae in Australia. Ecography 23:283-290

Crossland MR, Alford RA (1998) Evaluation of the toxicity of eggs, hatchlings and tadpoles of the introduced toad *Bufo marinus* (Anura: Bufonidae) to native Australian aquatic predators. Australian Journal of Ecology 23:129-137

Crowell KL (1968) Rates of competitive exclusion by the Argentine ant in Bermuda. Ecology 49:551-555

David P, Thébault E, Anneville O, Duyck P-F, Chapuis E, Loeuille N (2017) Impacts of invasive species on food webs: A review of empirical data. Advances in ecological research 56:1-60

Davidson D, Clark D, Jones T (2005) Gastral exocrine products of a myrmicine ant strongly overlap pygidial gland products of Dolichoderinae. Insectes sociaux 52:305-308

Davis NE, O'dowd DJ, Green PT, Nally RM (2008) Effects of an alien ant invasion on abundance, behavior, and reproductive success of endemic island birds. Conservation Biology 22:1165-1176

DeFisher LE, Bonter DN (2013) Effects of invasive European fire ants (*Myrmica rubra*) on Herring Gull (*Larus argentatus*) reproduction. PloS one 8:e64185

Dejean A, Amiet J-L (1992) Un cas de myrmécophilie inattendu: la cohabitation de l'anoure *Kassina senegalensis* avec la fourmi *Megaponera foetens*. Alytes 10:31-36

Delibes M (2005) La naturaleza en peligro: causas y consecuencias de la extinción de especies. Destino

Dhondt AA, Adriaensen F, Matthysen E, Kempenaers B (1990) Nonadaptive clutch sizes in tits. Nature 348:723-725

Di Giacomo AS, Di Giacomo AG (2006) Observations of Strange ‐ tailed Tyrants (*Alectrurus risora*) and other grassland birds following army ants and armadillos. Journal of Field Ornithology 77:266-268

Diaz M, Abril S, Enríquez ML, Gómez C (2013) Assessment of the Argentine ant invasion management by means of manual removal of winter nests in mixed cork oak and pine forests. Biological Invasions:1-13

Díaz-Paniagua C, Fernández Zamudio R, Florencio M, García Murillo P, Gómez-Rodríguez C, Portheault A, Serrano L, Siljeström P (2010) Temporay ponds from

Doñana National Park: a system of natural habitats for the preservation of aquatic flora and fauna. Limnetica 29:41-58

Díaz-Paniagua C, Fernández-Zamudio R, Serrano L, Florencio M, Gómez-Rodríguez C, Sousa A, Sánchez Castillo P, García-Murillo P, Siljestrom P (2016) El sistema de lagunas temporales de Doñana, una red de hábitats acuáticos singulares. Naturaleza y Parques Nacionales. Serie técnica

Díaz-Paniagua C, Gómez-Rodríguez C, Portheault A, de Vries W (2005) Los anfibios de Doñana, Madrid, Spain

Díaz-Paniagua C, Gómez-Rodríguez C, Portheault A, De Vries W (2006) Distribución de los anfibios del Parque Nacional de Doñana en función de la densidad y abundancia de los hábitats de reproducción. Revista española de herpetología 20:17-30

Didham RK, Tylianakis JM, Gemmell NJ, Rand TA, Ewers RM (2007) Interactive effects of habitat modification and species invasion on native species decline. Trends in ecology & evolution 22:489-496

Drees BM (1994) Red imported fire ant predation on nestlings of colonial waterbirds. The Southwestern Entomologist 19:355-359

Duarte H, Tejedo M, Katzenberger M, Marangoni F, Baldo D, Beltrán JF, Martí DA, Richter ‐ Boix A, Gonzalez ‐ Voyer A (2012) Can amphibians take the heat? Vulnerability to climate warming in subtropical and temperate larval amphibian communities. Global Change Biology 18:412-421

Duellman WE, Trueb L (1994) Biology of amphibians. JHU press

Eisner T, Aneshansley D (2008) "Anting" in Blue Jays: evidence in support of a food-preparatory function. Chemoecology 18:197-203

Elton CS (1958) The ecology of invasions by plants and animals. Methuen, London 18

Erel O (2004) A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clinical biochemistry 37:277-285

Espadaler X, Gómez C (2003) The Argentine ant, *Linepithema humile*, in the Iberian Peninsula. Sociobiology 42:187-192

Estany-Tigerström D, Bas JM, Clavero M, Pons P (2013) Is the blue tit falling into an ecological trap in Argentine ant invaded forests? Biological invasions 15:2013-2027

Estany-Tigerström D, Bas JM, Pons P (2010) Does Argentine ant invasion affect prey availability for foliage-gleaning birds? Biological invasions 12:827-839

Eyer P-A, McDowell B, Johnson LN, Calcaterra LA, Fernandez MB, Shoemaker D, Puckett RT, Vargo EL (2018) Supercolonial structure of invasive populations of the tawny crazy ant *Nylanderia fulva* in the US. BMC Evolutionary Biology 18:209

Feener DH, Brown BV (1997) Diptera as parasitoids. Annual review of entomology 42:73-97

Fernández Fe (2003) Introducción a las hormigas de la región Neotropical. Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Bogotá, Colombia

Fisher RN, Suarez AV, Case TJ (2002) Spatial patterns in the abundance of the coastal horned lizard. Conservation Biology 16:205-215

Flores M, Lazo P, Campbell G, Simeone A (2017) Breeding status of the red-tailed tropicbird (*Phaethon rubricauda*) and threats to its conservation on Easter Island (Rapa Nui). Pacific Science 71:149-160

Fluker SS, Beardsley JW (1970) Sympatric associations of three ants: *Iridomyrmex humilis*, *Pheidole megacephala*, and *Anoplolepis longipes* in Hawaii. Annals of the Entomological Society of America 63:1290-1296

Font-de-Mora R (1923) Sobre la presencia de la hormiga argentina (*Iridomyrmex humilis* Mayr) en Valencia. Boletin de la real sociedad española de historia natural 23:77-78

Forstmeier W, Weiss I (2004) Adaptive plasticity in nest ‐ site selection in response to changing predation risk. Oikos 104:487-499

Frank ET, Schmitt T, Hovestadt T, Mitesser O, Stiegler J, Linsenmair KE (2017) Saving the injured: Rescue behavior in the termite-hunting ant *Megaponera analis*. Science Advances 3:e1602187

Freed P, Neitman K (1988) Note on predation on the endangered Houston toad, *Bufo houstonensis*. TEXAS ACAD SCI TEXAS TECH UNIV, LUBBOCK, TX 79401, pp. 454-456

Freidenfelds N, Robbins T, Langkilde T (2012) Evading invaders: the effectiveness of a behavioral response acquired through lifetime exposure. Behavioral Ecology 23:659-664

Galán P (2003) Anfibios y Reptiles del Parque Nacional de las Islas Atlánticas de Galicia. Faunística, Biología y Conservación. Ministerio de Medio Ambiente, Madrid, Spain.

García-Mercet R (1923) Sobre la Icerya purchasi y la hormiga argentina. Bolletin De La Real Sociedad Española De Historia Natural 23:14-15

Garcia-Paris M (1990) Comportamiento de acecho en *Pelobates cultripes* (Cuvier, 1829). Revista Española de Herpetologia 4:45-49

GBIF, Global Biodiversity Information Facility. Available from https://www.gbif.org. Accessed 30th May 2018

Gibb H, Dunn RR, Sanders NJ, Grossman BF, Photakis M, Abril S, Agosti D, Andersen AN, Angulo E, Armbrecht I, Arnan X, Baccaro F, Bishop T, Boulay R, Brühl C, Castracani C, Cerdá X, Del Toro I, Delsinne T, Diaz M, Donoso D, Ellison A, Enriquez M, Fayle T, FeenerJr. D, Fisher B, Fisher R, Fitzpatrick M, Gómez C, Gotelli N, Gove A, Grasso D, Groc S, Guénard B, Gunawardene N, Heterick B, Hoffmann B, Janda M, Jenkins C, Kaspari M, Klimes P, Lach L, Laeger T, Lattke J, M L, Lessard J, Longino J, Lucky A, Luke S, Majer J, McGlynn T, Menke S, Mezger D, Mori A, Moses J, Munyai T, Pacheco R, Paknia O, Pearce-Duvet J, Pfeiffer M, Philpott S, Resasco J, Retana J, Silva R, Soroger D, Souza J, Suarez A, Tista M, Vasconcelos H, Vonshak M, Weiser M, Yates M, Parr C (2017) A global database of ant species abundances. Ecology 98:883-884

Gilardi JD, von Kugelgen K (1991) Bird/ant/acacia symbioses in a mature Neotropical forest. The Wilson bulletin 103:711-712

Giraud T, Pedersen JS, Keller L (2002) Evolution of supercolonies: the Argentine ants of southern Europe. Proceedings of the National Academy of Sciences 99:6075-6079

GISD, Global Invasive Species Database. Available from http://www.issg.org/database Accessed: 23th September 2018

Giuliano WM, Allen CR, Lutz RS, Demarais S (1996) Effects of red imported fire ants on northern bobwhite chicks. The Journal of wildlife management 60:309-313

GLAD, The Global Ants Database Available from http://globalants.org/

Glenn S, Holway D (2008) Consumption of introduced prey by native predators: Argentine ants and pit-building ant lions. Biological Invasions 10:273-280

Gomez-Mestre I, Tejedo M (2003) Local adaptation of an anuran amphibian to osmotically stressful environments. Evolution 57:1889-1899

Gómez-Mestre I, Tejedo M (2005) Adaptation or exaptation? An experimental test of hypotheses on the origin of salinity tolerance in *Bufo calamita*. Journal of Evolutionary Biology 18:847-855

González de la Vega JP (1988) Anfibios y reptiles de la provincia de Huelva. Juan Pablo González de la Vega

González-Varo JP, Biesmeijer JC, Bommarco R, Potts SG, Schweiger O, Smith HG, Steffan-Dewenter I, Szentgyörgyi H, Woyciechowski M, Vilà M (2013) Combined effects of global change pressures on animal-mediated pollination. Trends in ecology & evolution 28:524-530

Goodenough AE, Hart AG, Elliot S (2008a) Variation in offspring quality with cavity orientation in the great tit. Ethology Ecology & Evolution 20:375-389

Goodenough AE, Maitland DP, Hart AG, Elliot SL (2008b) Nestbox orientation: a species-specific influence on occupation and breeding success in woodland passerines. Bird Study 55:222-232

Gordon DM, Heller NE (2014) The invasive Argentine ant *Linepithema humile* (Hymenoptera: Formicidae) in Northern California reserves: from foraging behavior to local spread. Myrmecol News 19:103-110

Gosler A (1993) The great tit. Hamlyn

Gosner KL (1960) A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16:183-190

Gotelli NJ (1996) Ant community structure: effects of predatory ant lions. Ecology 77:630-638

Gove AD, Majer JD, Rico-Gray V (2009) Ant assemblages in isolated trees are more sensitive to species loss and replacement than their woodland counterparts. Basic and Applied Ecology 10:187-195

Gozlan RE, St-Hilaire S, Feist SW, Martin P, Kent ML (2005) Biodiversity: disease threat to European fish. Nature 435:1046

Grimes L (1973) The breeding of Heuglin's Masked Weaver and its nesting association with the red weaver ant. Ostrich 44:170-175

Haemig PD (1996) Interference from ants alters foraging ecology of great tits. Behavioral Ecology and Sociobiology 38:25-29

Haemig PD (1999) Predation risk alters interactions among species: competition and facilitation between ants and nesting birds in a boreal forest. Ecology Letters 2:178-184

Haemig PD (2001) Symbiotic nesting of birds with formidable animals: a review with applications to biodiversity conservation. Biodiversity & Conservation 10:527-540

Hagman M, Phillips BL, Shine R (2009) Fatal attraction: adaptations to prey on native frogs imperil snakes after invasion of toxic toads. Proceedings of the Royal Society of London B: Biological Sciences 276:2813-2818

Haight KL (2006) Defensiveness of the fire ant, *Solenopsis invicta*, is increased during colony rafting. Insectes sociaux 53:32-36

Harrell FE, Dupont C (2017) Hmisc: Harrell Miscellaneous

Hefetz A, Blum MS (1978) Biosynthesis of formic acid by the poison glands of formicine ants. Biochimica et Biophysica Acta 543:484-496

Hefetz A, Lloyd HA (1983) Identification of new components from anal glands of *Tapinoma simrothi* pheonicium. Journal of Chemical Ecology 9:607-613

Helanterä H, Strassmann JE, Carrillo J, Queller DC (2009) Unicolonial ants: where do they come from, what are they and where are they going? Trends in Ecology & Evolution 24:341-349

Heller NE (2004) Colony structure in introduced and native populations of the invasive Argenine ant, *Linepithema humile*. Insectes Sociaux 51:378-386

Heller NE, Ingram KK, Gordon DM (2008) Nest connectivity and colony structure in unicolonial Argentine ants. Insectes Sociaux 55:397-403

Helms JA, Godfrey AP, Ames T, Bridge ES (2016) Are invasive fire ants kept in check by native aerial insectivores? Biology Letters. pp. 20160059

Hemp C, Dettner K (1997) Morphology and chemistry of mesothoracic glands in anthicid beetles (Coleoptera: Anthicidae). Entomologia generalis 22:97-108

Hierro JL, Maron JL, Callaway RM (2005) A biogeographical approach to plant invasions: the importance of studying exotics in their introduced and native range. Journal of ecology 93:5-15

Hobbs RJ, Mooney HA (2000) Invasive species in a changing world: the interactions between global change and invasiveness. Invasive alien species. pp. 310-331

Hodar JA, Camacho I (1991) La alimentación de *Hyla meridionalis* (Boettger, 1874) en una población reproductora del sureste de la Península. Rev. Esp. Herp 5:15-22.

Hoffmann BD, Luque GM, Bellard C, Holmes ND, Donlan CJ (2016) Improving invasive ant eradication as a conservation tool: a review. Biological Conservation 198:37-49

Hoffmann M, Hilton-Taylor C, Angulo A, Böhm M, Brooks TM, Butchart SH, Carpenter KE, Chanson J, Collen B, Cox NA (2010) The impact of conservation on the status of the world's vertebrates. Science 330:1503-1509

Hölldobler B, Wilson EO (1990) The ants. Harvard University Press

Holway DA (1999) Competitive mechanisms underlying the displacement of native ants by the invasive Argentine ant. Ecology 80:238-251

Holway DA, Lach L, Suarez AV, Tsutsui ND, Case TJ (2002) The causes and consequences of ant invasions. Annual Review of Ecology and Systematics 33:181-233

Holway DA, Suarez AV (2004) Colony-structure variation and interspecific competitive ability in the invasive Argentine ant. Oecologia 138:216-222

Holway DA, Suarez AV (2006) Homogenization of ant communities in mediterranean California: The effects of urbanization and invasion. Biol. Conser. 127(3):319-326

Holway DA, Suarez AV, Case TJ (1998) Loss of intraspecific aggression in the success of a widespread invasive social insect. Science 282(5390):949-952

Hooper-Bui L, Rust M, Reierson D (2004) Predation of the endangered California Least Tern, *Sterna antillarum browni* by the southern fire ant, *Solenopsis xyloni* (Hymenoptera, Formicidae). Sociobiology 43:401-418

Huang Y, Zhu S, Tan D, Zeng L, Wang L, Xu Y (2016) Aggression of *Solenopsis invicta* towards other ants is not affected by colony rafting. Asian Myrmecol 8:1-4

Hulme PE, Bacher S, Kenis M, Klotz S, Kühn I, Minchin D, Nentwig W, Olenin S, Panov V, Pergl J (2008) Grasping at the routes of biological invasions: a framework for integrating pathways into policy. Journal of Applied Ecology 45:403-414

Isacch JP, Barg M (2002) Are bufonid toads specialized ant-feeders? A case test from the Argentinian flooding pampa. Journal of Natural History 36:2005-2012

Ito F, Okaue M, Ichikawa T (2009) A note on prey composition of the Japanese treefrog, *Hyla japonica*, in an area invaded by Argentine ants, *Linepithema* *humile*, in Hiroshima prefecture, western Japan (Hymenoptera: Formicidae). Myrmecological News 12:35-39

IUCN, International Union for Conservation of Nature. https://www.iucn.org/es Accessed 9th November 2018

IUCN Red List (v.2017-3) www.iucnredlist.org. Accessed 30th May 2018

Jackson TN, Koludarov I, Ali SA, Dobson J, Zdenek CN, Dashevsky D, Masci PP, Nouwens A, Josh P, Goldenberg J (2016) Rapid radiations and the race to redundancy: An investigation of the evolution of Australian elapid snake venoms. Toxins 8:309

Jäntti A, Suorsa P, Hakkarainen H, Sorvari J, Huhta E, Kuitunen M (2007) Within territory abundance of red wood ants *Formica rufa* is associated with the body condition of nestlings in the Eurasian treecreeper *Certhia familiaris*. Journal of Avian Biology 38:619-624

Janzen DH (1969) Birds and the ant × acacia interaction in Central America, with notes on birds and other myrmecophytes. Condor 71:240-256

Jeschke JM, Bacher S, Blackburn TM, Dick JT, Essl F, Evans T, Gaertner M, Hulme PE, Kühn I, Mrugała A (2014) Defining the impact of non ‐ native species. Conservation Biology 28:1188-1194

Jolly CJ, Shine R, Greenlees MJ (2016) The impacts of a toxic invasive prey species (the cane toad, *Rhinella marina*) on a vulnerable predator (the lace monitor, *Varanus varius*). Biological Invasions 18:1499-1509

Jourdan H, Sadlier R, Bauer A (2001) Little fire ant invasion (*Wasmannia auropunctata*) as a threat to New Caledonian lizards: Evidences from a sclerophyll forest (Hymenoptera: Formicidae). . Sociobiology 38:283-301

Judson OP, Bennett AT (1992) ‘Anting’as food preparation: formic acid is worse on an empty stomach. Behavioral Ecology and Sociobiology 31:437-439

Kahn Ce (2005) The Merck veterinary manual. 9th ed. Merck Hardcover, Philadelphia USA

Karvonen J, Orell M, Rytkönen S, Broggi J, Belda E (2012) Population dynamics of an expanding passerine at the distribution margin. Journal of avian biology 43:102-108

Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. Trends in Ecology & Evolution 17:164-170

Kenis M, Auger-Rozenberg M-A, Roques A, Timms L, Péré C, Cock MJ, Settele J, Augustin S, Lopez-Vaamonde C (2009) Ecological effects of invasive alien insects. Biological Invasions 11:21-45

Krushelnycky PD, Hodges CS, Medeiros AC, Loope LL (2001) Interaction between the Hawaiian dark-rumped petrel and the Argentine ant in Haleakala National Park, Maui, Hawaii. Studies in Avian Biology 22:243-246

Krushelnycky PD, Loope LL, Reimer NJ (2005) The ecology, policy, and management of ants in Hawaii. Proceedings of the Hawaiian Entomological Society 37:1-25

Kumschick S, Gaertner M, Vilà M, Essl F, Jeschke JM, Pyšek P, Ricciardi A, Bacher S, Blackburn TM, Dick JT, Evans T, Hulme P, Kühn I, Mrugala A, Pergl J, Rabitsch W, Richardson D, Sendek A, Winter M (2015) Ecological impacts of alien species: quantification, scope, caveats, and recommendations. Bioscience 65:55-63

Kurz-Besson C, Otieno D, Do Vale RL, Siegwolf R, Schmidt M, Herd A, Nogueira C, David TS, David JS, Tenhunen J (2006) Hydraulic lift in cork oak trees in a savannah-type Mediterranean ecosystem and its contribution to the local water balance. Plant and Soil 282:361-378

Laakkonen J, Fisher RN, Case TJ (2001) Effect of land cover, habitat fragmentation and ant colonies on the distribution and abundance of shrews in southern California. Journal of Animal Ecology 70:776-788

Lach L, Hooper-Bui LM (2010) Consequences of ant invasion. In: Lach L, Parr CL and Abbott KL (eds) Ant Ecology. Oxford University Press, New York, pp. 261-286

Lach L, Parr CL, Abott KL (2010) Ant ecology. Oxford University Press

Lachaud J-P, Pérez-Lachaud G (2012) Diversity of species and behavior of hymenopteran parasitoids of ants: a review. Psyche: A Journal of Entomology 2012:24 pages

Lambrechts MM, Schatz B (2014) Ants and Paridae share nesting boxes in continental Mediterranean habitat. Folia Zoologica 63:63-66

Lambrechts MM, Schatz B, Bourgault P (2008) Interactions between ants and breeding Paridae in two distinct Corsican oak habitats. Folia Zoologica 57:264-268

Langkilde T (2009) Invasive fire ants alter behavior and morphology of native lizards. Ecology 90:208-217

Langkilde T, Freidenfelds NA (2010) Consequences of envenomation: red imported fire ants have delayed effects on survival but not growth of native fence lizards. Wildlife Research 37:566-573

Langkilde T, Thawley CJ, Robbins TR (2017) Behavioral adaptations to invasive species: benefits, costs, and mechanisms of change. Advances in the Study of Behavior 49:199-235

LeBrun E, Tillberg C, Suarez A, Folgarait P, Smith C, Holway D (2007) An experimental study of competition between fire ants and Argentine ants in their native range. Ecology 88:63-75

LeBrun EG, Jones NT, Gilbert LE (2014) Chemical warfare among invaders: a detoxification interaction facilitates an ant invasion. Science:1245833

Lemm J (1997) Predation of captive reptiles in southern California by the introduced Argentine ant, Linepithema humile. Bulletin of the Association of Reptilian and Amphibian Veterinarians 7:4-5

Lenth RV (2016) Least-Squares Means: The R Package lsmeans.

Letnic M, Webb JK, Shine R (2008) Invasive cane toads (*Bufo marinus*) cause mass mortality of freshwater crocodiles (*Crocodylus johnstoni*) in tropical Australia. Biological Conservation 141:1773-1782

Li Y, Ke Z, Wang S, Smith GR, Liu X (2011) An exotic species is the favorite prey of a native enemy. PLoS ONE 6:e24299-1-10

Lieberburg I, Kranz PM, Seip A (1975) Bermudian ants revisited: the status and interaction of *Pheidole megacephala* and *Iridomyrmex humilis*. Ecology 56:473-478

Ligon RA, Siefferman L, Hill GE (2012) Invasive ants alter foraging and parental behaviors of a native bird. Ethology 118:858-866

Lindström J (1999) Early development and fitness in birds and mammals. Trends in Ecology & Evolution 14:343-348

Liu H-W, Lu Y-Y, Wang W-K, Chen L (2017) Whole body solvent soak gives representative venom alkaloid profile from *Solenopsis invicta* (Hymenoptera: Formicidae) workers. Florida Entomologist 100:522-527

Lizana M, Ciudad MJ, Pérez-Mellado V (1986) Uso de los recursos tróficos en una comunidad ibérica de anfibios. Rev Esp Herpetol 1: 207-271

Lizana M, Pérez-Mellado V (1990) Analysis of the structure of an amphibian community in the central system of Spain. Herpetol J 1:435-446

Llewelyn JS, Phillips BL, Shine R (2009) Sublethal costs associated with the consumption of toxic prey by snakes. Austral Ecology 34:179-184

Lockwood JL (1999) Using taxonomy to predict success among introduced avifauna: relative importance of transport and establishment. Conservation Biology 13:560-567

Long AK, Knapp DD, Mccullough L, Smith LL, Conner LM, Mccleery RA (2015) Southern toads alter their behavior in response to red-imported fire ants. Biological Invasions 17:2179-2186

López Jurado L (1982) Estudios sobre el sapo corredor (*Bufo calamita*) en el Sur de España. Doñana Acta Vertebrata 9:71-84

Lorrain A, Graham BS, Popp BN, Allain V, Olson RJ, Hunt BP, Potier M, Fry B, Galván-Magaña F, Menkes CE (2015) Nitrogen isotopic baselines and implications for estimating foraging habitat and trophic position of yellowfin tuna in the Indian and Pacific Oceans. Deep Sea Research Part II: Topical Studies in Oceanography 113:188-198

Lowe S, Browne M, Boudjelas S, De Poorter M (2000) 100 of the world's worst invasive alien species: a selection from the global invasive species database. Invasive Species Specialist Group Auckland,, New Zealand

Luque GM, Bellard C, Bertelsmeier C, Bonnaud E, Genovesi P, Simberloff D, Courchamp F (2013) The 100th of the world’s worst invasive alien species. Biological Invasions 16:981-985

Mack MC, D'Antonio CM (1998) Impacts of biological invasions on disturbance regimes. Trends in Ecology & Evolution 13:195-198

Mack RN, Simberloff D, Mark Lonsdale W, Evans H, Clout M, Bazzaz FA (2000) Biotic invasions: causes, epidemiology, global consequences, and control. Ecological Applications 10:689-710

Maerz JC, Karuzas JM, Madison DM, Blossey B (2005) Introduced invertebrates are important prey for a generalist predator. Diversity and Distributions 11:83-90

Majer J, Nichols O (1998) Long ‐ term recolonization patterns of ants in Western Australian rehabilitated bauxite mines with reference to their use as indicators of restoration success. Journal of Applied Ecology 35:161-182

Marshall MR, Cooper RJ (2004) Territory size of a migratory songbird in response to caterpillar density and foliage structure. Ecology 85:432-445

Martins M (1907) Une fourmi terrible envahissant l’Europe (*Iridomyrmex humilis* Mayr). Brotéria, Series Zoología 6:101-102

Matsui S, Kikuchi T, Akatani K, Horie S, Takagi M (2009) Harmful effects of invasive yellow crazy ant *Anoplolepis gracilipes* on three land bird species of Minami-daito Island. Ornithological Science 8:81-86

McCord JM, Fridovich I (1969) Superoxide dismutase: an enzymatic function for erythrocuprein (hemocuprein). J Biol Chem 244:6049-6055

McCue MD (2010) Starvation physiology: reviewing the different strategies animals use to survive a common challenge. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 156:1-18

McGlynn TP (1999) The worldwide transfer of ants: geographical distribution and ecological invasions. Journal of Biogeography 26:535-548

McKinney ML, Lockwood JL (1999) Biotic homogenization: a few winners replacing many losers in the next mass extinction. Trends in ecology & evolution 14:450-453

Mejías MA, Wingate DB, Madeiros JL, Wiersma YF, Robertson GJ (2017) Nest-cavity selection and nesting success of Bermudian white-tailed tropicbirds (*Phaethon lepturus catesbyi*). The Wilson Journal of Ornithology 129:586-599

Meyerson LA, Mooney HA (2007) Invasive alien species in an era of globalization. Frontiers in Ecology and the Environment 5:199-208

Miaud C, Sanuy D, Avrillier J-N (2000) Terrestrial movements of the natterjack toad Bufo calamita (Amphibia, Anura) in a semi-arid, agricultural landscape. Amphibia Reptilia 21:357-370

Mitrus S, Hebda G, Wesołowski T (2016) Cohabitation of tree holes by ants and breeding birds in a temperate deciduous forest. Scandinavian Journal of Forest Research 31:135-139

Moen DS, Wiens JJ (2017) Microhabitat and climatic niche change explain patterns of diversification among frog families. The American Naturalist 190:29-44

Mollot G, Pantel J, Romanuk T (2017) The effects of invasive species on the decline in species richness: a global meta-analysis. Advances in Ecological Research. Elsevier, pp. 61-83

Monaghan P (2008) Early growth conditions, phenotypic development and environmental change. Philosophical Transactions of the Royal Society B: Biological Sciences 363:1635-1645

Monaghan P, Metcalfe NB, Torres R (2009) Oxidative stress as a mediator of life history trade‐offs: mechanisms, measurements and interpretation. Ecology letters 12:75-92

Monzó C, Juan-Blasco M, Pekár S, Mollá Ó, Castañera P, Urbaneja A (2013) Pre-adaptive shift of a native predator (Araneae, Zodariidae) to an abundant invasive ant species (Hymenoptera, Formicidae). Biological invasions 15:89-100

Mooney HA, Cleland EE (2001) The evolutionary impact of invasive species. Proceedings of the National Academy of Sciences 98:5446-5451

Moore RP, Robinson WD (2004) Artificial bird nests, external validity, and bias in ecological field studies. Ecology 85:1562-1567

Moranz RA, Debinski DM, Winkler L, Trager J, McGranahan DA, Engle DM, Miller JR (2013) Effects of grassland management practices on ant functional groups in central North America. Journal of insect conservation 17:699-713

Morgenstern D, King GF (2013) The venom optimization hypothesis revisited. Toxicon 63:120-128

Morrison LW (2000) Mechanisms of interspecific competition among an invasive and two native fire ants. Oikos 90:238-252

Müller-Schärer H, Schaffner U, Steinger T (2004) Evolution in invasive plants: implications for biological control. Trends in Ecology & Evolution 19:417-422

Murray KA, Verde Arregoitia LD, Davidson A, Di Marco M, Di Fonzo MM (2014) Threat to the point: improving the value of comparative extinction risk analysis for conservation action. Global Change Biology 20:483-494

Myers PE, Allen CR, Birge HE (2014) Consuming fire ants reduces northern bobwhite survival and weight gain. Journal of Agricultural and Urban Entomology 30:49-58

Naef-Daenzer B (1994) Radiotracking of great and blue tits: new tools to assess territoriality, home-range use and resource distribution. Ardea-Wageningen 82:335-335

Naef‐Daenzer B, Widmer F, Nuber M (2001) Differential post‐fledging survival of great and coal tits in relation to their condition and fledging date. Journal of Animal Ecology 70:730-738

Naples VL (1999) Morphology, evolution and function of feeding in the giant anteater (*Myrmecophaga tridactyla*). Journal of Zoology 249:19-41

Ness JH, Mooney KA, Lach L (2010) Ants as mutualists. In: Lach L, Parr CL and Abbott KL (eds) Ant Ecology. Oxford University Press, New York, pp. 97-114

Newell W, Barber TC (1913) The Argentine ant. Bulletin Bureau of Entomology, U.S. Department of Agriculture 122:1-98

Nilsson SG (1984) The evolution of nest-site selection among hole-nesting birds: the importance of nest predation and competition. Ornis Scandinavica:167-175

Oi DH, Drees BM (2009) Fire ant IPM. In: Radcliffe EB, Hutchison WD and Cancelado RE (eds) Integrated pest management: concepts, tactics, strategies and case studies. Cambridge University Press,

Oksanen J, Blanchet GF, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2017) Vegan: Community Ecology Package.

Oliver JA (1955) The natural history of North American amphibians and reptiles.

Orivel J, Dejean A (2001) Comparative effect of the venoms of ants of the genus *Pachycondyla* (Hymenoptera: Ponerinae). Toxicon 39:195-201

Orrock JL, Danielson BJ (2004) Rodents balancing a variety of risks: invasive fire ants and indirect and direct indicators of predation risk. Oecologia 140:662-667

Packer JG, Meyerson LA, Richardson DM, Brundu G, Allen WJ, Bhattarai GP, Brix H, Canavan S, Castiglione S, Cicatelli A (2017) Global networks for invasion science: benefits, challenges and guidelines. Biological Invasions 19:1081-1096

Papillion AM, Hooper-Bùi LM, Strecker RM (2011) Flooding increases volume of venom sac in *Solenopsis invicta* (Hymenoptera: Formicidae). Sociobiology 57:301-308

Passera L (1994) Characteristics of tramp species. In: Williams DF (ed) Exotic Ants: biology, impact and control of introduced species. Boulder: Westview Press, pp. 23-43

Pasteels JM, Grégoire J-C, Rowell-Rahier M (1983) The chemical ecology of defense in arthropods. Annual review of entomology 28:263-289

Pavan M (1950) Potere insetticida della iridomirmecina e significato della sostanza nella biologia di *Iridomyrmex humilis* Mayr (Formica argentina). La Ricerca Scientifica 20:1835–1855

Pavan M (1951) Sull'attivita insetticida della iridomirmecina. Mem Soc Ent It 30:107–132

Pavan M (1952a) Iridomyrmecin as insecticide. Transactions of the 9th International Congress of Entomology, Amsterdam, August 17-24, 1951. Amsterdam, pp. 321-327

Pavan M (1952b) Primo contributo sperimentale allo studio farmacologico della iridomirmecina. Archives internationales de pharmacodynamie et de therapie 89:223-228

Pavan M (1952c) Suggli antibiotici di origine animale. I. Sintesi delle conoscenze attuali. Istituto di anatomia comparata dell'Università di Pavia:195-208

Pavan M (1952d) Suggli antibiotici di origine animale. II. Ricerche personali. Bollettino dell'Istituto sieroterapico milanese 31:232-245

Pavan M, Nascimbene A (1948a) Studi sugli antibiotici di origine animale. I. Su un principio antibiotico di *Iridomyrmex pruinosus humilis* Mayr. (nota prev.). Boll Soc Med Chir Pavia 62:193–197

Pavan M, Nascimbene A (1948b) Studi sugli antibiotici di origine animale. X. Nuovi risultati sulla iridomirmecina. Boll Soc Med Chir Pavia 62:295-298

Pavan M, Ronchetti G (1955) Studi sulla morfologia esterna e anatomia interna dell'operaia di *Iridomyrmex humilis* Mayr e ricerche chimiche e biologiche sulla iridomirmecina. Atti Soc It Sc Nat 94:379-477

Pechenik JA (2006) Larval experience and latent effects—metamorphosis is not a new beginning. Integrative and Comparative Biology 46:323-333

Pedersen EK, Grant WE, Longnecker MT (1996) Effects of red imported fire ants on newly-hatched northern bobwhite. The Journal of Wildlife management 60:164-169

Pedersen JS, Krieger MJB, Vogel V, Giraud T, Keller L (2006) Native supercolonies of unrelated individuals in the invasive Argentine ant. Evolution 60:782-791

Pejchar L, Mooney HA (2009) Invasive species, ecosystem services and human well-being. Trends in ecology & evolution 24:497-504

Pekár S, Mayntz D (2014) Comparative analysis of the macronutrient content of Central European ants (Formicidae): Implications for ant-eating predators. Journal of Insect Physiology 62:32-38

Persson L (1985) Asymmetrical competition: are larger animals competitively superior? The American Naturalist 126:261-266

Peterson BL, Kus BE, Deutschman DH (2004) Determining nest predators of the least bell's vireo through point counts, tracking stations, and video photography. Journal of Field Ornithology 75:89-95

Phillips B, Shine R (2007) When dinner is dangerous: toxic frogs elicit species-specific responses from a generalist snake predator. The American Naturalist 170:936-942

Philpott SM, Greenberg R, Bichier P (2005) The influence of ants on the foraging behavior of birds in an agroforest. Biotropica 37:468-471

Pianka ER, Parker WS (1975) Ecology of horned lizards: a review with special reference to *Phrynosoma platyrhinos*. Copeia:141-162

Pierce JL, Kostova T, Dirks KT (2003) The state of psychological ownership: Integrating and extending a century of research. Review of general psychology 7:84-107

Pintor LM, Byers JE (2015) Do native predators benefit from non-native prey? Ecology Letters 18:1174-1180

Plentovich S, Hebshi A, Conant S (2009) Detrimental effects of two widespread invasive ant species on weight and survival of colonial nesting seabirds in the Hawaiian Islands. Biological invasions 11:289-298

Plentovich S, Russell T, Fejeran CC (2018) Yellow crazy ants (*Anoplolepis gracilipes*) reduce numbers and impede development of a burrow-nesting seabird. Biological Invasions 20:77-86

Pons P, Bas JM, Estany-Tigerström D (2010) Coping with invasive alien species: the Argentine ant and the insectivorous bird assemblage of Mediterranean oak forests. Biodiversity and conservation 19:1711-1723

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703-718

Pyšek P (1998) Is there a taxonomic pattern to plant invasions? Oikos 82:282-294

Pyšek P, Jarošík V, Hulme PE, Pergl J, Hejda M, Schaffner U, Vilà M (2012) A global assessment of invasive plant impacts on resident species, communities and ecosystems: the interaction of impact measures, invading species' traits and environment. Global Change Biology 18:1725-1737

Pyšek P, Richardson DM, Pergl J, Jarošík V, Sixtova Z, Weber E (2008) Geographical and taxonomic biases in invasion ecology. Trends in Ecology & Evolution 23:237-244

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

R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria,

Rankin DT, Clark CJ, Rankin EEW (2018) Hummingbirds use taste and touch to discriminate against nectar resources that contain Argentine ants. Behavioral Ecology and Sociobiology 72:44

Ransom TS (2012) Comparison of direct, indirect, and ecosystem engineering effects of an earthworm on the red‐backed salamander. Ecology 93:2198-2207

Read GW, Lind NK, Oda CS (1978) Histamine release by fire ant (*Solenopsis*) venom. Toxicon 16:361-367

Recuero E (2014) Sapo de espuelas - *Pelobates cultripes*. In: Salvador A and Martínez Solano I (eds) Enciclopedia Virtual de los Vertebrados Españoles. Museo Nacional de Ciencias Naturales, Madrid,

Ricciardi A (2007) Are modern biological invasions an unprecedented form of global change? Conservation Biology 21:329-336

Ricciardi A, Hoopes MF, Marchetti MP, Lockwood JL (2013) Progress toward understanding the ecological impacts of nonnative species. Ecological Monographs 83:263-282

Richardson D, Pyšek P (2008) Fifty years of invasion ecology–the legacy of Charles Elton. Diversity and Distributions 14:161-168

Richardson DM (2011) Invasion science. the roads travelled and the roads ahead. In: Richardson DM (ed) Fifty years of invasion ecology: the legacy of Charles Elton. Blackwell Publishing Ltd, pp. 397-407

Riddick EW, Brown AE, Chauhan KR (2008) *Harmonia axyridis* adults avoid catnip and grapefruit-derived terpenoids in laboratory bioassays. Bulletin of Insectology 61:81

Ridlehuber KT (1982) Fire ant predation on wood duck ducklings and pipped eggs. The Southwestern Naturalist 27:222-222

Robbins TR, Freidenfelds NA, Langkilde T (2013) Native predator eats invasive toxic prey: evidence for increased incidence of consumption rather than aversion-learning. Biological invasions 15:407-415

Robbins TR, Langkilde T (2012) The consequences of lifetime and evolutionary exposure to toxic prey: changes in avoidance behaviour through ontogeny. Journal of evolutionary biology 25:1937-1946

Rödel MO, Braun U (1999) Associations between anurans and ants in a West African Savanna (Anura: Microhylidae, Hyperoliidae, and Hymenoptera: Formicidae). Biotropica 31:178-183

Rodríguez S, Noordwijk AJ, Álvarez E, Barba E (2016) A recipe for postfledging survival in great tits *Parus major*: be large and be early (but not too much). Ecology and evolution 6:4458-4467

Rowles AD, O'Dowd DJ (2007) Interference competition by Argentine ants displaces native ants: implications for biotic resistance to invasion. Biological Invasions 9:73-85

Rowles AD, O'Dowd DJ (2009) New mutualism for old: indirect disruption and direct facilitation of seed dispersal following Argentine ant invasion. Oecologia 158:709-716

Rowles AD, Silverman J (2009) Carbohydrate supply limits invasion of natural communities by Argentine ants. Oecologia 161(1):161-171

Rull V (2018) El Antropoceno. CSIC, Consejo Superior de Investigaciones Científicas

Sala OE, Chapin FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NL, Sykes MT, Walker BH, Walker M, Wall DH (2000) Global biodiversity scenarios for the year 2100. science 287:1770-1774

Salo P, Korpimäki E, Banks PB, Nordström M, Dickman CR (2007) Alien predators are more dangerous than native predators to prey populations. Proceedings of the Royal Society of London B: Biological Sciences 274:1237-1243

Sánchez-García D, Cuesta-Segura AD, Herraiz JA, Trigos-Peral G, García-García F, Catarineu C, Arcos-González J, Fernández-Martínez JA (2017) Checklist of the ants of the Iberian Peninsula and Balearic Islands. In: Abril S (ed) XII Iberian Meeting of Myrmecology. Poster communication. Iberomyrmex, pp. 74

Sanders NJ, Gotelli NJ, Heller NE, Gordon DM (2003) Community disassembly by an invasive species. Proceedings of the National Academy of Sciences 100:2474-2477

Sanders NJ, Suarez AV (2011) Elton's insights into the ecology of ant invasions: lessons learned and lessons still to be learned. In: Richardson DM (ed) Fifty Years of Invasion Ecology: The Legacy of Charles Elton. Blackwell Publishing Ltd, pp. 239-251

Saporito RA, Donnelly MA, Spande TF, Garraffo HM (2012) A review of chemical ecology in poison frogs. Chemoecology 22:159-168

SAS Institute Inc. (2008) SAS/STAT® 9.2 User's Guide. SAS Inst, Cary, NC. USA

SAS Institute Inc. (2011) SAS/STAT® 9.3 User's Guide. SAS Inst, Cary, NC. USA

Sax DF, Stachowicz JJ, Brown JH, Bruno JF, Dawson MN, Gaines SD, Grosberg RK, Hastings A, Holt RD, Mayfield MM (2007) Ecological and evolutionary insights from species invasions. Trends in Ecology & Evolution 22:465-471

Schlesinger WH (2006) Global change ecology. Trends in ecology & evolution 21:348-351

Schlüter A, Regős J (1996) The tadpole of *Lithodytes Lineatus*-with note on the frogs resistance of leaf-cutting ants (Amphibia: Leptodactylidae). Stuttgarter Beiträge zur Naturkunde Serie A:1-4

Schmidt JO (1986) Chemistry, pharmacology, and chemical ecology of ant venoms. In: Pied T (ed) Venoms of the Hymenoptera: biochemical, pharmacological and behavioural aspects. Academic Press, London, pp. 425-508

Schmidt JO (2009) Venom. Encyclopedia of Insects (Second Edition). Elsevier, pp. 1028-1031

Schmidt PJ, Sherbrooke WC, Schmidt JO (1989) The detoxification of ant (*Pogonomyrmex*) venom by a blood factor in horned lizards (*Phrynosoma*). Copeia 1989:603-607

Schmitz PE (1897) As formigas da Madeira. Annaes de Sciencias Naturaes IV

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nature methods 9:671

Seebens H, Blackburn TM, Dyer EE, Genovesi P, Hulme PE, Jeschke JM, Pagad S, Pyšek P, van Kleunen M, Winter M, Ansong M, Arianoutsou M, Bacher S, Blasius B, Brockerhoff E, Brundu G, Capinha C, Causton C, Celesti-Grapow L, Dawson W, Dullinger S, Economo E, Fuentes N, Guénard B, Jäger H, Kartesz J, Kenis M, Kühn I, Lenzner B, Liebhold A, Mosena A, Moser D, Nentwig W, Nishino M, Pearman D, Pergl J, Rabitsch W, Rojas-Sandoval J, Roques A, Rorke S, Rossinelli S, Roy H, Scalera R, Schindler S, Štajerová K, Tokarska-Guzik B, Walker K, Ward D, Yamanaka T, Essl F (2018) Global rise in emerging alien species results from increased accessibility of new source pools. Proceedings of the National Academy of Sciences 115:E2264-E2273

Sergio F, Newton I, Marchesi L, Pedrini P (2006) Ecologically justified charisma: preservation of top predators delivers biodiversity conservation. Journal of Applied Ecology 43:1049-1055

Seymour MA (2007) Effect of red imported fire ant (*Solenopsis invicta* Buren) on the nesting success of northern bobwhite (*Colinus virginianus* L.). Louisiana State University, Baton Rouge, pp. 129

Shackleton RT, Shackleton CM, Kull CA (2018) The role of invasive alien species in shaping local livelihoods and human well-being: a review. Journal of environmental management

Sherbrooke WC, Schwenk K (2008) Horned lizards (*Phrynosoma*) incapacitate dangerous ant prey with mucus. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology 309:447-459

Shine R (2010) The ecological impact of invasive cane toads (*Bufo marinus*) in Australia. The Quarterly Review of Biology 85:253-291

Shine R (2014) A review of ecological interactions between native frogs and invasive cane toads in Australia. Austral Ecology 39:1-16

Shirihai H, Svensson L (2018) Handbook of Western Palearctic birds: passerines. Helm, London,

Shorter J, Rueppell O (2012) A review on self-destructive defense behaviors in social insects. Insectes Sociaux 59:1-10

Sih A, Bolnick DI, Luttbeg B, Orrock JL, Peacor SD, Pintor LM, Preisser E, Rehage JS, Vonesh JR (2010) Predator–prey naïveté, antipredator behavior, and the ecology of predator invasions. Oikos 119:610-621

Simberloff D (2011) Charles Elton: neither founder nor siren, but prophet. In: Richardson DM (ed) Fifty years of invasion ecology: the legacy of Charles Elton. Blackwell Publishing Ltd, pp. 11-24

Simberloff D, Martin J-L, Genovesi P, Maris V, Wardle DA, Aronson J, Courchamp F, Galil B, García-Berthou E, Pascal M (2013) Impacts of biological invasions: what's what and the way forward. Trends in ecology & evolution 28:58-66

Smith T, Shugart H (1987) Territory size variation in the ovenbird: the role of habitat structure. Ecology 68:695-704

Sockman KW (1997) Variation in life-history traits and nest-site selection affects risk of nest predation in the California gnatcatcher. The Auk 114:324-332

Spring D, Croft L, Kompas T (2017) Look before you treat: increasing the cost effectiveness of eradication programs with aerial surveillance. Biological invasions 19:521-535

Stake MM, Cimprich DA (2003) Using video to monitor predation at Black-capped Vireo nests. The Condor 105:348-357

StatSoft Inc (2002) Statistica for Windows (computer program manual). In: Tulsa Ohwsc (ed).

Stjernman M, Råberg L, Nilsson J-Å (2008) Long-term effects of nestling condition on blood parasite resistance in blue tits (*Cyanistes caeruleus*). Canadian Journal of Zoology 86:937-946

Stoddard HL (1931) The bobwhite quail: its habits, preservation and increase, New York

Strategic Plan 2020-Aichi targets https://www.cbd.int/sp/targets. Accessed 9th November 2018

Strong AM (2000) Divergent foraging strategies of two Neotropical migrant warblers: implications for winter habitat use. The Auk 117:381-392

Suarez AV, Bolger DT, Case TJ (1998) Effects of fragmentation and invasion on native ant communities in coastal southern California. Ecology 79(6):2041-2056

Suarez AV, Case TJ (2002) Bottom-up effects on persistence of a specialist predator: Ant invasions and horned lizards. Ecological Applications 12:291-298

Suarez AV, Holway DA, Case TJ (2001) Patterns of spread in biological invasions dominated by long-distance jump dispersal: insights from Argentine ants. Proceedings of the National Academy of Sciences 98:1095-1100

Suarez AV, Holway DA, Tsutsui ND (2008) Genetics and behavior of a colonizing species: The invasive argentine ant. American Naturalist 172:S72-S84

Suarez AV, Richmond JQ, Case TJ (2000) Prey selection in horned lizards following the invasion of Argentine ants in southern California. Ecological Applications 10:711-725

Suarez AV, Tsutsui ND, Holway DA, Case TJ (1999) Behavioral and genetic differentiation between native and introduced populations of the Argentine ant. Biol. Invasions 1(1):43-53

Suarez AV, Yeh P, Case TJ (2005) Impacts of Argentine ants on avian nesting success. Insectes Sociaux 52:378-382

Takahashi N, Touyama Y, Kameyama T, Ito F (2018) Effect of the Argentine ant on the specialist myrmecophilous cricket *Myrmecophilus kubotai* in western Japan. Entomological Science

Therneau TM (2015) coxme: Mixed Effects Cox Models. R package version 2.2-5. 2.38 edn.,

Tillberg CV, Holway DA, LeBrun EG, Suarez AV (2007) Trophic ecology of invasive Argentine ants in their native and introduced ranges. Proceedings of the National Academy of Sciences 104:20856-20861

Tomalski M, Blum M, Jones T, Fales H, Howard D, Passera L (1987) Chemistry and functions of exocrine secretions of the ants *Tapinoma melanocephalum* and *T. erraticum*. Journal of chemical ecology 13:253-263

Touchard A, Aili SR, Fox EGP, Escoubas P, Orivel J, Nicholson GM, Dejean A (2016) The biochemical toxin arsenal from ant venoms. Toxins 8:30

Touyama Y, Ihara Y, Ito F (2008) Argentine ant infestation affects the abundance of the native myrmecophagic jumping spider *Siler cupreus* Simon in Japan. Insectes Sociaux 55:144-146

Tsutsui ND, Suarez AV, Holway DA, Case TJ (2000) Reduced genetic variation and the success of an invasive species. Proceedings of the National Academy of Sciences of the United States of America 97:5948-5953

Twardochleb LA, Novak M, Moore JW (2012) Using the functional response of a consumer to predict biotic resistance to invasive prey. Ecological Applications 22:1162-1171

Tylianakis JM, Didham RK, Bascompte J, Wardle DA (2008) Global change and species interactions in terrestrial ecosystems. Ecology Letters 11:1351-1363

Uemura M, Perkins LE, Zalucki MP, Cribb BW (2017) Predator–prey interaction between greenhead ants and processionary caterpillars is mediated by chemical defence. Animal Behaviour 129:213-222

Ugelvig LV, Drijfhout FP, Kronauer DJ, Boomsma JJ, Pedersen JS, Cremer S (2008) The introduction history of invasive garden ants in Europe: integrating genetic, chemical and behavioural approaches. BMC biology 6:1-14

Valverde JA (1967) Estructura de una comunidad mediterránea de vertebrados terrestres. Consejo Superior de Investigaciones Científicas, Madrid, Spain.

van Oudenhove L, Boulay R, Lenoir A, Bernstein C, Cerdá X (2012) Substrate temperature constrains recruitment and trail following behavior in ants. Journal of Chemical Ecology 38:802-809

Van Wilgenburg E, Torres CW, Tsutsui ND (2010) The global expansion of a single ant supercolony. Evolutionary Applications 3:136-143

Vanderklift MA, Ponsard S (2003) Sources of variation in consumer-diet δ 15 N enrichment: a meta-analysis. Oecologia 136:169-182

Varela AI, Luna N, Luna-Jorquera G (2018) Assessing potential Argentine ant recruitment to pipping eggs in the red-tailed tropicbird on Rapa Nui (Easter Island). Emu-Austral Ornithology:1-5

Vázquez VA (1999) Ecología trófica de una población suburbana de Sapillo pintojo ibérico (*Discoglossus galganoi* Capula *et al*., 1985) en el noroeste de la Península Ibérica. Tesina de Licenciatura, Universidad de A Coruña, Spain.

Venables WN, Ripley BD (2002) Modern Applied Statistics with S. Springer, New York

Vilà M, Espinar JL, Hejda M, Hulme PE, Jarošík V, Maron JL, Pergl J, Schaffner U, Sun Y, Pyšek P (2011) Ecological impacts of invasive alien plants: a meta‐analysis of their effects on species, communities and ecosystems. Ecology letters 14:702-708

Vitousek PM (1994) Beyond global warming: ecology and global change. Ecology 75:1861-1876

Vitousek PM, D'Antonio C, Loope LL, Westbrooks R (1996) Biological invasions as global environmental change. American Scientist 84:468-478

Vogel V, Pedersen JS, d'Ettorre P, Lehmann L, Keller L (2009) Dynamics and genetic structure of Argentine ant supercolonies in their native range. Evolution 63:1627-1639

Vogel V, Pedersen JS, Giraud T, Krieger MJB, Keller L (2010) The worldwide expansion of the Argentine ant. Diversity and Distributions 16:170-186

Wallis AC, Smith RL, Beard KH (2016) Temporal foraging patterns of nonnative coqui frogs (*Eleutherodactylus coqui*) in Hawaii. Journal of Herpetology 50:582-588

Walters A, Mackay DA (2005) Importance of large colony size for successful invasion by Argentine ants (Hymenoptera: Formicidae): evidence for biotic resistance by native ants. Austral Ecology 30:395-406

Wanger TC, Wielgoss AC, Motzke I, Clough Y, Brook BW, Sodhi NS, Tscharntke T (2011) Endemic predators, invasive prey and native diversity. Proc Biol Sci 278:690-4

Wells KD (2010) The ecology and behavior of amphibians. University of Chicago Press

Welzel KF, Lee SH, Dossey AT, Chauhan KR, Choe D-H (2018) Verification of Argentine ant defensive compounds and their behavioral effects on heterospecific competitors and conspecific nestmates. Scientific Reports 8:1477

Wetterer JK, Porter SD (2003) The little fire ant, *Wasmannia auropunctata*: distribution, impact, and control. Sociobiology 42:1-42

Wetterer JK, Wetterer AL (2006) A disjunct Argentine ant metacolony in Macaronesia and southwestern Europe. Biological Invasions 8:1123-1129

Wetterer JK, Wetterer AL, Hebard E (2001) Impact of the Argentine ant, *Linepithema humile* on the native ants of Santa Cruz Island, California. Sociobiology 38:709-722

Wetterer JK, Wild AL, Suarez AV, Roura-Pascual N, Espadaler X (2009) Worldwide spread of the Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae). Myrmecol. News 12:187-194

Wheeler JW, Olagbemiro T, Nash A, Blum MS (1977) Actinidine from the defensive secretions of dolichoderine ants. Journal of Chemical Ecology 3:241-244

Wickham H (2009) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York,

Wiebe KL, Gow EA (2013) Choice of foraging habitat by northern flickers reflects changes in availability of their ant prey linked to ambient temperature. Ecoscience 20:122-130

Wigger E, Kuhn-Nentwig L, Nentwig W (2002) The venom optimisation hypothesis: a spider injects large venom quantities only into difficult prey types. Toxicon 40:749-752

Wild AL (2004) Taxonomy and distribution of the Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae). Annals of the Entomological Society of America 97:1204-1215

Wilson EO (2003) Pheidole in the New World: a dominant, hyperdiverse ant genus. Harvard University Press

Wilson EO (2016) Half Earth: Our Planet's fighting for live. W. W. Norton & Company

Wu C-S, Gomez-Mestre I, Kam Y-C (2012) Irreversibility of a bad start: early exposure to osmotic stress limits growth and adaptive developmental plasticity. Oecologia 169:15-22

Young BE, Kaspari M, Martin TE (1990) Species-specific nest selection by birds in ant-acacia trees. Biotropica 22:310-315

Zee J, Holway D (2006) Nest raiding by the invasive Argentine ant on colonies of the harvester ant, *Pogonomyrmex subnitidus*. Insectes Sociaux 53:161-167